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A STUDY OF AN OUTBREAK OF INFLUENZA B IN ROCHESTER, NEW YORK

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Influenza B virus was first isolated independently by Magill[9] and Francis[2] in 1940, and since then it has been recognized as the cause of outbreaks of influenza both in the United States and abroad. As Horsfall[6] pointed out, influenza is an inexactly defined symptom-complex. It is now accepted that the exact diagnosis of influenza is most satisfactorily established by the demonstration of at least a 4-fold rise in antibody titer as determined by the Hirst test,[5] or by isolation and identification of the causative virus. Epidemic influenza may be caused by various strains of either influenza A or B virus, but in some outbreaks neither virus has been incriminated in as many as 30% of the cases.[7] Furthermore, it has been reported that both A and B viruses may occur simultaneously in the same population, or, rarely, in the same individual.[7,10,13] Gordon[3] has shown that there are quantitative differences as well as antigenic relationships between strains of influenza B virus. That a strain of influenza B virus may be associated with fatal bacterial infection has been reported by Himmelweit[4] who isolated the virus from a patient who quickly succumbed to pneumonia. Postmortem examination showed destruction of the epithelium of the tracheobronchial tree, pulmonary hemorrhage and necrosis, as well as paucity of cellular reaction. The lungs contained enormous numbers of staphylococci. Nigg, Ekland, Wilson and Crowley[10] reported a 50% mortality in 8 patients (average age over 45 years) who developed pneumonia in the course of influenza B.

Because of the prospect of an epidemic of influenza in this community, it was planned to study a group of patients should an outbreak occur. The purposes of the investigation were not only to attempt to determine the causative agents, but also to appraise clinically the value of sulfonamide prophylaxis in diminishing the frequency of bacterial complications. Hence a plan to treat alternate cases with sulfadiazine was projected.

The present report is based upon the findings in 46 patients who were admitted to the Strong Memorial and Rochester Municipal Hospitals from Dec. 10 to 18, 1945, inclusive. Actually about 80 patients with a similar pattern of disease were observed during the months of November and December, but we have limited the number of cases considered here for the following reasons. First,

lacking positive etiologic evidence, there is no certain way of differentiating influenza B from many other acute respiratory illnesses. Secondly, before December 10, most of the patients suspected of having influenza seemed to represent sporadic cases from the community at large. Thirdly, a sudden influx of cases between December 10 and 18, inclusive, was largely from a somewhat isolated group of young men living on the River Campus of the University of Rochester. These patients represented only the more severely ill cases from that campus. Fourthly, the admission rate dropped abruptly after December 18, and from then on only apparently sporadic cases were seen. Hence, a precipitous onset in a detached and fairly homogeneous group characterizes the bulk of the cases reported. Of the 46 patients, 37 were male, and 9 were female. Their ages ranged from 17 to 62 years; five-sixths of the group were 18 to 22 years of age. Thirty-four were students at the River Campus, 10 were personnel of the hospitals, and the remaining 2 were from the community at large. The student body and the hospital personnel were vaccinated against influenza A and B between December 15 and 19; 8 of the patients were members of these vaccinated groups. The latter had symptoms of from 1 to 3 days duration that were thought, in most cases, to be due to actual infection rather than to have resulted from the vaccine.

The clinical study of the patient included, in addition to the routine history and physical examination, white blood cell counts and differential counts, bacterial cultures of the blood and nasopharynx, serologic tests for cold hemagglutinins and heterophil antibodies. From 4 patients the nasopharyngeal washings were inoculated in embryonated hen's eggs and intranasally in mice in an attempt to isolate a virus. From these same 4 patients and 5 additional ones, serum was obtained on admission, and again 3 or 4 weeks later, for the performance of the Hirst test. Twenty-three patients had chest roentgenograms. Because of the rapidity of admissions and the shortage of personnel, all of the projected studies were not performed in every case.

Symptoms and Signs. Twelve patients had symptoms for a day before admission to the hospital, 18 for 2 days, 10 for 3 days, and 8 for 4 to 7 days. Three-fourths of the group had only low grade fever (not over 38° C.). Ten patients had temperatures of 39° C. or higher, with relative bradycardia and normal respiratory rates. The clinical symptoms and signs in the 46 patients under consideration are grouped together in Table 1 in order of decreasing frequency. Except for 1 individual who had had a cough and

Table 1.—Incidence of Symptoms and Signs in 46 Cases of Influenza B

Dry cough	37	Injected nasal mucosa	30
Headache	32	Suffusion of eyes	13
Chilliness	29	Flushed facies	10
Fever	27	Cervical lymphadenopathy	7
Sore throat	27	Generalized lymphadenopathy	5
Coryza	23	Perspiring freely	3
Myalgia	18	Injected tympani	3
Malaise	14	Pulmonary râles	4
Anorexia	12	Pulmonary rhonchi	3
Substernal pain	12	Palpable submaxillary glands	2
Ocular myalgia	11	Prostration	2
Asthenia	8	Sinus tenderness	1
Vomiting	6	Palpable spleen	1
Photophobia	4	Papular rash	1
Hoarseness	3	Nuchal rigidity	1
Otalgia	2	Lethargy	1
Diarrhea	2	Delirium	1
Abdominal cramps	2	Lacrimation	1
Injected pharynx	37		

slight loss of weight for a month, all were in good health prior to the onset of symptoms. A typical case is described:

Case 1. V. V. (216850), a 19 year old student from the River Campus, was admitted on Dec. 12, 1945, with the complaints of sore throat, non-productive coughing, generalized aching, malaise, coryza, retrobulbar pain, retrosternal pain on coughing and fever of 2 days duration. His temperature was 40.6° C., pulse 110 and respirations 20. He appeared acutely ill. The facies were flushed and the conjunctivæ suffused. The pharynx was injected, and there was catarrhal otitis media bilaterally. Râles were audible in the region of the right upper lobe. On admission, the white blood cell count was 3600 of which 73% were neutrophils, 24% lymphocytes and 2% monocytes. Roentgenogram of the chest showed an increase of the bronchovascular markings. Blood culture was negative, while alpha-hemolytic colonies, *Staph. aureus* and a non-hemolytic streptococcus appeared in the nasopharyngeal culture. Whereas the sheep cell titer was 1:16, and the cold hemagglutination test negative, the Hirst test showed that during convalescence he developed a significant rise in titer of antibodies against influenza B virus. He was treated with 37 gm. of sulfadiazine over a period of 7 days, although he exhibited fever for only 3 days. The white blood cell count increased to 6200 during the period of administration of sulfadiazine. Following discharge on the 8th day, he experienced asthenia for 2 days, but felt well thereafter.

Laboratory Findings. As may be seen in Table 2, the admission white blood cell counts tended to be low. Differential counts revealed from 60 to 90% neutrophils; as a general rule, however, there was no significant shift in the differential formula. *Strep. pyogenes* was found in the throat cultures of 4 patients; other bacteria isolated by aërobic culture of nasopharyngeal swabs on blood agar plates are listed in Table 3. Blood cultures were done on 31 patients. From 3 of these, bacteria of possible significance were isolated, in 2 instances *Strep. viridans* and in the third, a non-hemolytic streptococcus. From only 1 of the 3 cases was there available blood serum taken both early in the disease and during convalescence. The serums from this patient were tested for agglutinins against the organism isolated from the culture of his blood (*Strep. viridans*) according to a technique used by Finland.[1] Serum taken at the onset of influenza agglutinated the strain to a titer of 1:160, while serum taken 3 weeks later showed an identical titer of agglutinins. It is, therefore, impossible to assess any significance to the finding of a streptococcus in this patient's blood. Furthermore, the failure to demonstrate an increase in antibodies against it during convalescence would make one look askance upon the other 2 instances in which the streptococci were obtained by blood culture.

Tests for cold hemagglutinins were done during the 1st week in 19 patients. The test was negative in 11, and insignificant titers were found in 8, the highest being

Table 2.—Admission White Blood Cell Counts

Range	No. patients	Range	No. patients
2000 to 4900	19	8000 or more	3
5000 to 7900	21	Not done	3

Table 3.—Nasopharyngeal Flora

Bacteria	No. patients	Bacteria	No. patients
Staph. albus	13	*H. influenzæ*	4
H. parainfluenzæ	11	*Staph. albus hemolyticus*	3
Strep. viridans	11	*N. catarrhalis*	3
"Diphtheroids"	9	*Staph. aureus hemolyticus*	3
Non-hemolytic streptococci	8	*M. tetragenus*	1
Staph. aureus	6	Not done	18
Strep. pyogenes	4		

1:20. However, when checked about 1 month later, the latter's serum had a titer of 1:160. This patient's hospital course of 6 days was unremarkable, but 2 weeks later he had fever, cough, and headache and remained in bed for a week. This person may have had influenza, but clinical relapse and rising cold hemagglutinin titer during convalescence suggested the possibility of primary atypical pneumonia. Serums obtained, after an interval of 3 or 4 weeks, from 7 additional patients, whose initial level of cold hemagglutinins had been determined, did not reveal any rise in titer.

Heterophile antibody tests were done in 17 instances. The results were negative in 3, insignificant in 13, and positive to 1:256 dilution in 1 patient.

Case 2. A student from the River Campus had chilliness, fever, headache, backache, malaise, sore throat, coryza and dry cough for 2 days prior to admission. The temperature was 38.5° C., pulse 108 and respirations 21. He, too, appeared toxic and had injection of the nasopharynx. The white blood cell count was 4700 with 74% neutrophils, 18% lymphocytes and 6% monocytes. The chest Roentgen ray revealed increased bronchovascular markings in the right lower lobe. *Strep. viridans* was obtained on culture of the blood. The Hirst test was negative. He was treated with 22 gm. of sulfadiazine over a 6 day period during which the leukopenia became more marked. When the sheep cell titer was repeated 1 month later, it was 1:128. He had been well in the interval.

Another student with a negative Hirst test and an equivocal titer of agglutinins for sheep erythrocytes (1:32) had a total white blood cell count of 4000 per mm.; 48% of the cells were lymphocytes. His course was uneventful, and he did not report for interview several weeks later. He may, however, have had infectious mononucleosis rather than influenza. Mention should be made of the fact that sporadic cases of infectious mononucleosis had been observed in the student body during the few weeks preceding the outbreak of influenza. Two patients were thought to have had only systemic reactions to vaccinations for influenza A and B, rather than influenza.

All the remaining patients were considered to have had influenza B. As is shown in Table 4, Hirst tests (4) were performed on 9 of these selected at random. During convalescence none developed an increase in circulating antibodies against influenza A virus. Two were entirely negative, 1 of these was from the individual known to have had infectious mononucleosis, the other from the student described above who was suspected of having

Table 4.—Results of Hirst Tests Done With Acute Phase and Convalescent Serums of 9 Patients Selected at Random

	Influenza A				Influenza B	
	PR 8		WS		Lee	
Patients	a	c	a	c	a	c
M.	32	32	32	32	16	16
S.	64	64	32	64	32	32
V. V.	64	64	64	32	8	32
W.	16	16	16	16	8	32
B.	16	32	16	16	8	64
D.	32	16	32	16	32	128
H.	8	8	32	32	32	128
T.	32	32	128	128	32	128
F.	16	16	16	16	8	32
Controls:						
Ferret–PR 8*		512		128		8
Ferret–Lee*		8		32		256
Normal ferret*		0		64		0

a, Acute phase serum; c, convalescent serum.
* Serums furnished by Dr. Thomas Francis, Ann Arbor, Mich.
Positive tests are those with 4-fold or greater rise of titer.

infectious mononucleosis. Altogether 7 patients exhibited significant rises in circulating antibodies against influenza B virus. Inasmuch as none of these serums had been obtained from patients who were vaccinated against influenza A and B, the etiology of the outbreak of influenza-like symptoms, in the majority of the patients, appeared to be influenza B virus. Influenza virus was not isolated in the 4 instances attempted.

Of the 23 patients who had chest roentgenograms, 6 were negative, but 17 showed increased bronchovascular markings. One also showed minimal evidence of infiltration in the left upper lobe. All of these were negative when repeated either before discharge from the hospital, or 3 weeks later.

Results of Prophylactic Treatment With Sulfadiazine. Twenty patients were treated symptomatically. As a group they exhibited fever for an average period of 1.5 days, and were hospitalized for an average of 4.1 days. One of the 20 had meningismus, and another sinusitis. The latter also had questionable signs of early bronchopneumonia, and was given 400,000 units of penicillin over a 3 day period.

In accordance with the plan to treat alternate cases prophylactically with sulfadiazine, in order to determine its value in diminishing the incidence and severity of complications of influenza, those patients selected were given on admission 4 gm. of the drug by mouth. This was followed by 1 gm. every 4 hours until the temperature had been normal for at least 2 or 3 days. In addition, when a complication appeared, some of the previously untreated controls were then started on the same dose of sulfadiazine, except for the 1 individual treated with penicillin as mentioned above. Altogether, 26 patients received from 10 to 36 gm. of the drug. Of these, 22 were treated prophylactically, 2 because of otitis media, and 1 each because of bronchitis or laryngitis. This group of patients manifested fever for 2.2 days, and was hospitalized for an average of 6.6 days. The longer hospital stay was due, in part, to the policy of continuing sulfadiazine therapy until 2 or 3 days of normal temperature had been observed. Six patients developed microscopic hematuria which necessitated discontinuance of the drug; 2 had traces of albuminuria. The renal complications could be attributed in every instance to unanticipated confinement of the patient in a hot room of low humidity. The effect of the sulfonamide therapy on the white blood cell count, already depressed by influenza, was not remarkable.

Thirty-one patients from the River Campus were interviewed 4 or 5 weeks after discharge in order to evaluate further the effectiveness of sulfadiazine prophylaxis. Almost all of these young men had traveled widely during the Christmas holiday, many on crowded trains. Of the 31, none had developed pneumonia; 16 denied any residual symptoms or complications; they felt well after discharge from the hospital. Seven of those treated with sulfadiazine had temporary asthenia, as against 2 of the control group. Persistent coughing was noted by 4 in the untreated group, and by only 2 of those who were treated with sulfadiazine.

There was 1 other patient, however, from the hospital personnel, who had prophylactic sulfadiazine for 3 days and was discharged after 10 days. He had to be re-admitted a day later because of acute, purulent otitis media. *Strep. pyogenes* was cultured from the ear. He had a white blood cell count of 1900.

Discussion. Since significant rises in antibody titer against influenza B virus were demonstrated in 7 patients selected at random, we feel that influenza B virus was responsible for the epidemic studied. The syndromes of influenza A and influenza B have been described as indistinguishable in groups as well as in individuals.[8] Some emphasize the more common occurrence of sweating, giddiness, nausea, vomiting and substernal chest pain in influenza B infection.[12] We do not feel that our data permits this distinction, ex-

cept, perhaps, with respect to substernal pain which occurred in 12 of our patients. The occasional similarity as witnessed in the present study, of the symptom-complexes of those known to have either influenza B, infectious mononucleosis, or primary atypical pneumonia is striking. In-as-much as neither influenza virus nor immunologic evidence of infection due to it has been found in a substantial number of individuals in some previously reported epidemics of influenza, consideration of other etiologic agents is warranted. In the present investigation, limited largely to young adults, influenza B was largely a self-limiting respiratory illness with few bacterial complications, the most frequent being otitis media. On the basis of the data presented, no benefit can be accorded the use of sulfadiazine in preventing bacterial complications in patients with influenza B infection. The data are, of course, insufficient to permit a final opinion.

Shope has suggested a concept of widespread pre-seeding of the causative agent to account for the rapidity of appearance of new cases of both swine influenza and human influenza in the course of epidemics. The same author has also suggested the possible rôle of meteorologic factors in evoking outbreaks of the disease.[11] In Chart 1 we have plotted the frequency of occurrence of our cases, dated at the onset of symptoms, against the daily variations in temperature in Rochester, N. Y., for December 1945. It may be of interest to note that the cases appeared during rather wide fluctuations in temperature over a short period of time.

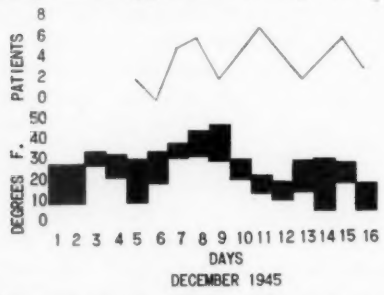

FIG. 1.— Comparison of onset of symptoms with fluctuations in temperature in Rochester. The blocked areas represent range of temperature in ° F. in the Rochester area; the heavy line, the variations in numbers of patients who had onset of symptoms on those days.

Summary and Conclusions. During a 9 day period in December 1945, 46 patients (chiefly young men from a college campus) were admitted to the hospital with an acute respiratory illness; 2 were thought to have reactions to influenza A and B vaccination; 2, infectious mononucleosis; 1, atypical pneumonia, and the remaining 41 to have influenza B. In 7 of the 41 selected at random, the Hirst test was positive for influenza B. Increased bronchovascular markings in chest Roentgen rays were seen in many. Seven developed minor complications, the commonest being otitis media. None had significant sequelæ. Twenty-six were treated with sulfadiazine prophylactically, or for complications. Although the total number of cases was too small to permit a final opinion, the incidence of complications, and the subsequent convalescence did not appear to be favorably influenced by sulfadiazine prophylaxis as compared with experience in an untreated control group.

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RELATIVE CLINICAL AND HEMATOLOGIC EFFECTS OF CONCENTRATED LIVER EXTRACT, SYNTHETIC FOLIC ACID AND SYNTHETIC 5-METHYL URACIL IN THE TREATMENT OF MACROCYTIC ANEMIAS IN RELAPSE*

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Because of the world-wide distribution of the macrocytic anemias, physicians everywhere have contemplated the best method for their detection and therapy. In his excellent book, which was published in 1855, Thomas Addison[1] of London described the clinical picture of one of the macrocytic anemias which he called pernicious anemia. Biermer, in 1872, in a lecture given before the Medical Society of Zurich, described this anemia in greater detail and called it "progressive" pernicious anemia. He pointed out that this condition in some way involved the digestive system.

The idea of taking mammalian liver internally goes back to antiquity in the mythology of many races. In 1926 Minot and Murphy[2] initiated the era of its modern usefulness in man when they showed that the oral administration of large doses of whole liver effected a remission of Addisonian pernicious anemia in relapse. Liver was soon tested in various types of macrocytic anemia and was found to be an effective hematopoietic substance in the treatment of the macrocytic anemia of pregnancy, of tropical sprue, of nontropical sprue, of nutritional deficiency, and of pernicious anemia.

It soon became apparent, however, that a potent extract from liver would be less difficult to administer than whole liver. A number of crude liver extracts were prepared, and within 2 years Cohn had developed his Fraction G.[3] This Fraction G became widely used in treating macrocytic anemias due to the absence or deficiency of erythrocyte maturation factor (EMF). EMF was considered to be the end-product of a reaction between the extrinsic factor, which was found in food, and the intrinsic factor, which was found in the secretions of the gastric mucosa. On the basis of this concept, which was fostered by Castle, it was possible to classify further the macrocytic anemias on the basis of intrinsic factor or extrinsic factor deficiency. Thus, Castle considers that the macrocytic anemia of pernicious anemia is characterized by a deficiency of intrinsic factor, while the macrocytic anemias of pregnancy, nutritional deficiency, and sprue are characterized by a deficiency of extrinsic factor. The intrinsic and extrinsic factor studies will not be discussed in this paper. With this concept in mind, many investigators directed their efforts toward the intrinsic factor, the extrinsic factor, or the EMF. Many fractions obtained from liver were hematologically active, but the great majority of them had no hematopoietic activity whatever. Many other substances such as brewers' yeast, hog stomach, kidney and brain were tried with success. Several of these substances had been known for many years to have hematopoietic properties, but none of them came up to the hematopoietic standards of concentrated liver extract. Because of the efficacy of concentrated liver extract in

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inducing remissions in the EMF deficiency anemias, it became the standard of those students who were constantly assaying new substances and new liver fractions. It soon was apparent that the various preparations themselves varied considerably, depending on the source of extraction, the method involved, and many other uncontrollable factors. It became obvious to some that liver might contain many substances that might be hematopoietically active. This concept was strengthened by the observations that some substance or substances were destroyed in the concentration of crude liver extract as determined by the hematopoietic response effected by both crude and concentrated liver extract.

Jacobson and SubbaRow[4] consider that there is no single intrinsic or extrinsic factor but that there are many factors that are necessary to produce a full, maximal hematologic response when the substances in question are given in minimal doses. In the fall of 1945, the authors and many other investigators were working on the problem. Realizing that liver extract was a mixture of many chemical substances, we knew that we were facing a difficult and tedious task. Throughout the years we had been studying fractions of reticulogen prepared by Dr. J. K. Cline, and we had tested the hemapoietic properties of many nutrients and synthetic substances. Some of these had slight activity, but most of them had none. We were also working with concentrates; 1 of these was folic acid concentrate which was furnished to us by Dr. E. A. Sharp. We had long known that a number of concentrated liver extracts which were of high potency in treating pernicious anemia contained only infinitesimal amounts of folic acid. Nevertheless, our working hypothesis was that a number of chemical molecules could probably effect hemopoiesis and that folic acid might well be one of these even if it were not the active one in concentrated liver extract. When folic acid became available in sufficient quantities for clinical testing, its effect on patients with macrocytic anemia was so dramatic that its efficacy was very quickly demonstrated.[5,6,7] Thus, for the first time it was possible to administer a pure synthetic compound to persons with the macrocytic anemia of pernicious anemia, of nutritional deficiency, of pregnancy, or tropical sprue, and of non-tropical sprue and to effect a hematologic remission. This discovery was of great importance for many reasons. Probably the most important is that its discovery has at long last placed investigations in the field of the macrocytic anemias in the realm of pure compound therapy. With the advent of pure compound therapy a step closer has been taken toward learning the etiology and pathogenesis of the disease.

The initial observations were difficult to evaluate in terms of past therapeutic performance because even though a pure synthetic compound was available it was necessary to assay it against and compare it to concentrated liver extract about which little is known in respect to the number and nature of the factors it contains. However, it has been conclusively established that folic acid is an effective hematopoietic substance in the treatment of all of the EMF deficiency anemias.[8,9,10] To date, there have been no negative reports concerning its use in these anemias. The hematologic response to folic acid is much the same as it is to liver extract. It has no effect on aplastic anemias, hypochromic anemia, primary thrombocytopenia, idiopathic leukopenia and leukemia.[10,11] Folic acid is also ineffective where liver extract is ineffective.

Once the efficiency of folic acid in the treatment of macrocytic anemia was established, a search was continued for other pure synthetic compounds of known structure. By March 1946,[12] the hematopoietic properties of 5-methyl uracil (thymine) were reported. This substance is one of the 4 nucleotides occurring in thymonucleic acid and is a basic cell constituent. We have since shown that synthetic 5-methyl uracil in daily oral doses of 15 gm. is effective in causing a remis-

sion in the macrocytic anemia of tropical sprue,[13] pernicious anemia[14] and nutritional macrocytic anemia.[15] Because of significant differences in the hematologic response of such patients to synthetic 5-methyl uracil and synthetic folic acid, it is considered important to report these differences. Since there are also certain minor differences in the response of patients with macrocytic anemia to synthetic folic acid and concentrated liver extract, it is also considered proper to report those differences. The neurologic aspects will not be discussed in this paper. It is fully realized that there are many hematopoietic compounds, but it has been our aim to assay pure compounds against pure compounds; and we believe that concentrated liver extract when compared with these other anti-anemic preparations probably contains the least number of different substances. From a controlled scientific point of view it is not desirable to compare a pure compound such as folic acid or 5-methyl uracil with an impure compound such as liver extract. The comparison is made because liver extract is the most widely used, the best known, and primarily because it is the most effective anti-anemic therapeutic agent we have at our disposal to date.

We are reporting on a total of 100 patients treated with concentrated liver extract, 24 patients treated with synthetic folic acid, and 14 patients treated with synthetic 5-methyl uracil. These patients had either true addisonian pernicious anemia, nutritional macrocytic anemia, or the macrocytic anemia of tropical sprue. Representative cases were chosen for sake of simplicity and detail. This report is concerned with a comparison of the hematopoietic and clinical effect of each of these 3 substances in 1 case of addisonian pernicious anemia in relapse and in 1 case of nutritional macrocytic anemia in relapse, and with the effect of synthetic folic acid in 1 case of sprue and of synthetic 5-methyl uracil in 1 case of sprue.

Selection of Patients. All the patients selected for study met the following basic criteria: (1) A mean corpuscular volume of 100 $c\mu$ or more. (2) A mean corpuscular hemoglobin of 32 $\mu\mu g$. or more. (3) A mean corpuscular hemoglobin concentration of 34% or more. (4) A color index of 1 or greater. (5) A red blood count of 2.5 million or less. (6) Megaloblastic arrest of the sternal bone marrow. (7) The patient must be untreated or he must not have been treated recently enough to interfere in any way with the evaluation of anti-anemic therapy.

In the selection of persons with pernicious anemia, an additional criterion was a histamine refractory achlorhydria and achylia which had remained unchanged over a period of 1 to 4 years during which time gastric analyses were done every 6 to 12 months. The selection of patients with nutritional macrocytic anemia was made by the use of the basic criteria, but in addition each patient selected must have free hydrochloric acid, rennin and pepsinogen in the gastric secretions after histamine stimulation as well as evidence of concomitant nutritive failure.

The selection of persons with tropical sprue was determined by the following criteria in addition to the basic criteria mentioned above: (1) There must be free hydrochloric acid and enzymes in the gastric secretions after histamine stimulation. (2) The single dose oral glucose tolerance curve must be flat. (3) The patient must have glossitis and diarrhea characterized by stools which show an increase in total fat and a preponderance of fatty acids as determined by chemical analysis. (4) There must be a weight loss of at least 30 pounds in the 6 months prior to study.

The 4 patients thus selected were hospitalized on each occasion prior to the institution of therapy. Careful medical and dietary histories as well as complete physical and neurologic examinations were done prior to treatment. During hospitalization the diet was rigidly controlled and contained no meat, meat products, fish or fowl and allowed only 1 egg and $\frac{1}{2}$ pint of

milk a day. The egg, as well as the vegetables, were cooked at high temperatures for a long period of time in an effort to destroy any extrinsic factor that might occur in these foods. Those patients who had tropical sprue were denied milk and eggs entirely in addition to the above dietary restrictions. Baseline clinical, hematologic and laboratory studies were performed as described elsewhere.[5,9] To our knowledge none of these patients had ever had a spontaneous remission. The baseline periods varied because treatment was always withheld until it appeared that the patient was actually getting worse both clinically and hematologically.

When such a point was reached, therapy was instituted. The dosage of liver extract, folic acid and 5-methyl uracil was varied in almost each patient because at the time we were endeavoring to determine minimal and maximal doses. We have since learned that 0.5 cc. of concentrated liver extract (reticulogen),* 10 to 20 mg. of synthetic folic acid (Folvite),† and 15 gm. of synthetic 5-methyl uracil (thymine)‡ are practical for the average case. These amounts are in the average case about the minimal doses which will give a reticulocyte response that is not influenced by an increase, no matter how great, in the substance employed. It has also been determined that folic acid is effective when given parenterally or orally,[8] and for that reason the parenteral route was abandoned in these studies.

The concentrated liver extract used in these studies was kept at a temperature below 55° C. Immediately prior to administration, the required dose was removed from the chilled vial and the medication was injected into the right or left gluteal muscles. The site of injection was alternately changed each day. Synthetic folic acid and synthetic 5-methyl uracil were weighed on an analytical balance immediately prior to administration. These drugs were partially dissolved and suspended in $\frac{1}{4}$ to $\frac{1}{2}$ glass of water and administered orally.

Case Reports. CASE 1. J. B. (addisonian pernicious anemia). This 46 year old white married male was first seen by us on May 29, 1944, and was hospitalized on that date. At that time he had many complaints, the most prominent of which were dizziness, weakness, shortness of breath, cardiac palpitation, pedal edema, redness and soreness of the tongue, and tingling and numbness of the hands and feet. Physical examination done at that time revealed severe generalized pallor, a divergent strabismus of congenital origin, scars and fissuring of both angles of the mouth which were interpreted as evidence of old cheilosis, severe pyorrhea, severe redness of the tongue and complete atrophy of the lingual papillæ,+++ pitting edema of the legs and feet, hypesthesia to light touch and pin prick of a stocking type over the left foot, and diminished vibratory sense over the ankles and knees. Position sense was intact and there was slight calf muscle tenderness to pressure. There was no change in the reflexes, and there were no pathologic reflexes found. Laboratory studies revealed a histamine refractory achlorhydria and achylia and a negative blood Klein as well as negative urinalyses, stool analyses, chest plate, and gastro-intestinal Roentgen ray series. An icterus index was slightly elevated. Hematologic studies showed an erythrocyte count of 1.25 million, hemoglobin of 5.1 gm. (33%), a reticulocyte count of 1.8%, a leukocyte count of 3250, PCV of 16, MCV of 128, MCH of 40.8, and MCHC of 31.8. Sternal bone marrow showed arrest at the megaloblastic level. Table 1 shows the packed cell volume and blood indices determined on each occasion prior to the initiation of specific therapy.

The patient was treated with some success with 2 liver fractions obtained from reticulogen in this laboratory. He was discharged on June 28, 1944, in a somewhat improved condition.

He was followed in our clinic for almost 6 months and received no liver or other substances containing the so-called antianemic principle. By December 6, he had a recurrence of the symptoms enumerated

* Supplied by Eli Lilly & Co.
† Supplied by Lederle Laboratories, Inc.
‡ Supplied by Hoffmann-La Roche, Inc.

above and was hospitalized. Physical examination was essentially as recorded on his first hospitalization. The erythrocyte count was 1.69 million, hemoglobin 5.4 gm., and the leukocyte count 4950. The reticulocytes were 0.2%, and sternal bone marrow was characteristic of megaloblastic arrest. Gastric analysis performed after histamine stimulation showed achlorhydria and achylia. By December 9, the erythrocyte count was 1.39 million, the hemoglobin was 4.4 gm., the reticulocytes were 0.2%, and therapy was instituted. The patient received 1 cc. of reticulogen I.M. every day for a period of 6 days. As seen in Figure 1, a peak reticulocytosis of 37.3% was reached on the 6th day after therapy was initiated. Simultaneously with this reticulocytosis there was a striking upsurge in well-being and improvement in appetite. On Feb. 3, 1945, 57 days after therapy was initiated, the red blood cell count was 5.08 million and the hemoglobin 13.1 gm. It should be noted that this patient received a total of only 6 cc. of a potent liver extract and thereafter received no anti-anemic therapy. Concomitant with the remission in this patient's blood picture there was complete clinical improvement. The redness of his tongue disappeared and it became pale and repapillated. Table 2 shows in summary form the hematologic response to this treatment as well as to the other forms of treatment employed.

When he was discharged from the hospital, he felt and appeared strong and well. He was followed in our clinic for almost 9 months without receiving any specific anti-anemic therapy. Gradually he relapsed hematologically and clinically and was readmitted to the hospital on Sept. 22, 1945, with symptoms of severe glossitis, stomatitis, profound weakness, dyspnea, vertigo, tinnitus, anorexia and moderate to severe paresthesias of the hands and feet. Physical examination revealed essentially the same findings as were noted on the first admission in May of 1944. Laboratory studies showed a histamine refractory achlorhydria and achylia, an essentially normal gastro-intestinal tract as determined by Roentgen ray examination, and normal urinalyses and stool analyses. The erythrocyte count was 1.96 million, the hemoglobin 7.9 gm. (51%), the leukocyte count 4800, and reticulocytes 0.4%. Blood indices were compatible with a morphologic diagnosis of macrocytic hyperchromic anemia in relapse. Sternal bone marrow studies showed arrest of the marrow cells at the megaloblastic level. On Sept. 23, 1945, the red blood cell count was 1.64 million, the hemoglobin 7.1 gm., and therapy was instituted. The patient received 50 mg. of synthetic folic acid by mouth twice daily for a period of 20 days as shown in Figure 1. On the 5th day of this therapy a peak reticulocytosis of 19.2% was attained. Hemopoiesis was rapid and the patient was discharged on October 12, with a red blood cell count of 4.07 million, a hemoglobin of 9.8 gm. (64%), a leukocyte count of 9850, and a reticulocyte count of 1%. Clinically the patient responded equally as well as he did hematologically. At the end of a 65 day period his erythrocyte count had reached a level of 4.97 million, and his hemoglobin was 12.5 gm. Again it should be realized that this patient was under treatment for a period of only 20 days after which he received no specific anti-anemic therapy.

He was again followed in clinic and received no specific therapy for 3 months, and by the end of this time he had relapsed once more and had many of the symptoms he had at the time of each previous relapse. He was admitted to the hospital on March 14, 1946. Physical examination showed essentially the same abnormalities as were present on the 3 previous hospitalizations except that on this occasion there was hyperesthesia to pin prick of a glove type over both hands and wrists. Laboratory data revealed a histamine refractory achlorhydria and achylia, an icterus index of 13.4 units (normal 8 to 10 units), an essentially normal gastro-intestinal series, normal chest Roentgen ray, normal oral glucose tolerance test, and essentially normal urinalyses and stool analyses. Sternal bone marrow was again characteristic of megaloblastic arrest, and on the day of admission to the hospital the hematologic studies revealed a red cell count of 1.55 million, a hemoglobin of 6.2 gm. (40%), a white cell count of 3050, a reticulocyte count of 0.3%, PCV of 20, MCV of 129, MCH of 40, and MCHC of 31. By March 17, the baseline studies were completed, and therapy was instituted. The patient received 2 gm. of synthetic 5-methyl uracil 3 times a day orally for a period of 19 days. A peak reticulocytosis of 16% was reached on the 11th day

of this treatment; 21 days after therapy had been instituted the erythrocytes had shown an increase of 530,000 cells per c.mm. of peripheral blood, while the hemoglobin showed an increase of 3.1 gm. per 100 cc. of peripheral blood. Figure 1 shows well that the hematologic response in this man to 5-methyl uracil at this dose level was far below that obtained with folic acid or concentrated liver extract. It should be recalled, however, that the dose employed was not standard. The clinical response was also less dramatic. The patient's tongue remained essentially unchanged; and his appetite which had originally improved, became worse during subsequent treatment. Paresthesias of his hands remained unchanged. After treatment was discontinued, the erythrocytes failed to continue to maturate and relapse was rapid. Figure 1 shows that the maximum erythrocyte count attained was 2.39 million 21 days after therapy was instituted. The slow rate of increase is illustrated in the flat type of curve also shown in this figure.

Sternal bone marrow obtained on the day following the peak reticulocytosis was done during each course of therapy. The marrow was normoblastic and in a reactive state at this stage of treatment. It was impossible to differentiate these marrow preparations on the basis of the therapeutic substances employed. To all intents and purposes the bone marrow response was essentially identical to each form of therapy.

Case 2. E. S. (nutritional macrocytic anemia). This 75 year old white married male was seen by us for the first time on June 28, 1943, the date of hospitalization, with complaints of weakness, anorexia, cardiac palpitation, dyspnea, vertigo, soreness and redness of the mouth and the tongue, severe paresthesias and numbness of the hands and feet, burning of the soles of the feet, and soreness of the calf and thigh muscles bilaterally. He had a chronic intermittent diarrhea consisting of 3 to 6 loosely formed, brown-colored stools a day which were never characterized or described as being frothy, foamy, voluminous or foul-smelling. All of these symptoms were approximately of 1 year's duration during which time he had lost 15 to 20 pounds of weight. A diet history revealed a deficient intake of thiamine (B_1) and niacin. He had eaten no meat for the preceding 8 years and had eaten only small amounts of cooked liver occasionally during this period of time. Physical examinations revealed a severely pale man whose skin had a slightly yellow tinge. There were 2 large purpuric lesions over the left forearm and hand. His tongue was pale and edematous with many fissures over the dorsum, and there was evidence of old cheilosis bilaterally. Peripheral arteriosclerosis was present to a moderate degree. The neurologic examination revealed hyperesthesia to pin prick in the regions around both elbows. A stocking type hypesthesia to light touch was present over the lower extremities, and there was a reduction in vibratory sense of 30% in the arms and 80 to 90% in the legs bilaterally. Calf muscle and nerve trunk tenderness was exquisite. Position sense was intact. There were no pathologic reflexes present. The knee jerks and ankle jerks were hyperactive bilaterally; otherwise the reflexes were physiologic. Laboratory data revealed a negative blood Klein, an essentially normal gastro-intestinal Roentgen ray series and chest Roentgen ray, and normal urinalyses and stool analyses. A gastric analysis showed hypochlorhydria and the presence of rennin and pepsinogen after histamine stimulation. The icterus index was 16 units. The sternal bone marrow revealed arrest at the megaloblastic level. Hematologic studies showed an erythrocyte count of 670,000, a hemoglobin of 2.9 gm. (18%), and a leukocyte count of 1750. The patient remained in the hospital until March 11, 1944, during which time many substances were assayed for hematologic activity. The hematologic responses obtained with these substances are not within the scope of this report and consequently are deleted from Figure 2. Suffice it to say that the patient was discharged with a red blood count of 3.4 million and hemoglobin of 11.3 gm. on that date.

He was followed in clinic under non-specific therapy and gradually relapsed. He was readmitted to the hospital on Oct. 1, 1944. with the symptoms and signs as were described on his first admission. A diet history revealed an inadequate intake of thiamine (B_1) and niacin and a borderline adequacy of protein and vitamins A, B_2(G) and C. Laboratory studies showed a slightly elevated icterus index and the presence of free hydrochloric acid and enzymes in the gastric secretions after histamine stimulation. Blood studies on October 1 revealed a red blood

TABLE 1.—PACKED CELL VOLUME AND BLOOD INDICES OF CASE 1

Date	PCV	MCV	MCH	MCHC	Color index	Type of treatment
5/29/44	16	128 0	40 8	31 8	1 32	Liver fractions from reticulogen (Lilly)
12/6/44	17	100 5	31 9	31 7	1 60	Reticulogen
9/22/45	23	117 3	40 3	34 3	1 80	Synthetic folic acid
3/14/46	20	129 0	40 0	31 0	1 29	Synthetic 5-methyl uracil

TABLE 2.—HEMATOLOGIC RESPONSE OF CASE 1 (ADDISONIAN PERNICIOUS ANEMIA) TO CONCENTRATED LIVER EXTRACT, SYNTHETIC FOLIC ACID, AND SYNTHETIC 5-METHYL URACIL

	Erythrocytes (millions)			Hemoglobin (gm.)			Reticulocytes					
Date of initiation of therapy	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial % (on 1st day of therapy)	First day of rise	Day of peak	% at peak	Treatment and dose	No. days administered
12/9/44	1 39	2 94	5 08 (57)	4 4	7 0	13 1 (57)	0 2	4	6	37 3	Reticulogen, 1 cc. I.M. q.d.	6
9/23/45	1 64	2 51	4 97 (65)	7 1	9 0	12 5 (65)	0 2	4	5	19 2	Synthetic folic acid, 50 mg. b.i.d. orally	20
3/17/46	1 86	2 09	2 39 (21)	5 8	6 8	8 9 (21)	0 6	4	11	16 0	Synthetic 5-methyl uracil, 2 gm. t.i.d. orally	19

TABLE 3.—PACKED CELL VOLUME AND BLOOD INDICES OF CASE 2

Date	PCV	MCV	MCH	MCHC	Color index	Type of treatment
6/28/43	10	141 0	43 0	29 0	1 30	Various liver fractions from reticulogen and other preparations
10/1/44	14	125 0	37 5	30 0	1 20	Reticulogen
2/4/46	22	115 8	37 9	32 7	1 20	Folic acid
6/4/46	26	104 0	30 5	29 2	0 96	5-methyl uracil

count of 1.12 million, a hemoglobin of 4.2 gm. (27%), a white blood count of 3250, a reticulocyte count of 2.6%, and a PCV of 14. Blood indices were as follows: MCV 125, MCH 37.5, MCHC 30. The color index was 1.2. The packed cell volume and blood indices determined prior to therapy on each occasion are shown in Table 3. Sternal bone marrow obtained during baseline studies showed arrest at the megaloblastic level. By October 4, the red blood cell count was 1.03 million and the hemoglobin 4.2 gm., and therapy was instituted. The patient received 1 cc. of reticulogen I.M. every day for 15 days. The reticulocyte response was evident on the 3rd day of therapy, and a peak reticulocytosis of 21.4% was reached on the 8th day of treatment as shown in Figure 2 and Table 4. When the reticulocytes began to increase, the patient volunteered that he felt much better in many ways. His appetite improved; he regained his strength; the symptoms referrable to his glossitis and peripheral neuritis began to disappear. On November 30, 58 days after therapy was instituted, the erythrocyte count was 5.95 million and the hemoglobin was 12.7 gm. The patient felt well and strong and had only residual numbness of his extremities which was believed to be caused by arteriosclerosis.

During 1945 the patient had 2 relapses and was treated with both whole blood and concentrated liver extract, as well as with several other substances. The treatment of these relapses and subsequent responses have been deleted from Figure 2 for the sake of clarity and conciseness. The patient received no specific anti-anemic therapy after September 1945, and by November 28 his red cell count had reached a level of 4.17 million and the hemoglobin was 11.5 gm.

Thereafter he relapsed once more and was readmitted to the hospital on Feb. 4, 1946. He had a recurrence of all the symptoms described above. A diet history revealed a dietary very low in meat products and deficient in protein, iron and niacin, and of borderline adequacy in thiamine (B_1). The physical examination was essentially as determined on his first admission in 1943. Hematologic studies done on the day of admission showed a red blood count of 1.79 million, a hemoglobin of 6.9 gm. (44%), a white blood count of 4550, and a reticulocyte count of 0.1%. The packed cell volume and the blood indices are shown in Table 3. Gastric analysis done on this admission showed a histamine refractory achlorhydria and achylia. This was repeated after an interval of 2 days, and again a histamine refractory achlorhydria and achylia was obtained. Sternal bone marrow aspirated during the baseline studies was characteristic of megaloblastic arrest. An oral glucose tolerance test showed a slight lag period in absorption, but otherwise it was essentially normal. Urinalyses and stool analyses were essentially normal, and an icterus index obtained prior to therapy was 12.4 units. After satisfactory baseline studies were made, therapy was instituted. On that date, February 13, the red blood cell count was 1.46 million, the hemoglobin 4.3 gm., and the reticulocyte count 0.4%. The patient received 5 mg. of synthetic folic acid twice daily by mouth for a period of 12 days. The reticulocytes began to rise on the 5th day of this treatment, and a peak reticulocytosis of 60.4% was reached on the 8th day as is shown in Table 4. The patient's clinical response did not parallel the tremendous reticulocyte response. He did, however, experience the usual increase in well-being and increase in appetite along with the reticulocytosis. His glossitis improved steadily but at a slightly slow rate. His peripheral neuritis remained essentially unchanged. On the 41st day after treatment had been instituted the red blood count was 3.81 million and the hemoglobin was 11.2 gm. (71%). This was the highest erythrocyte level reached after this course of therapy.

The patient was again followed in the clinic and received no specific anti-anemic therapy. By June 4, 1946, he had relapsed and was admitted to the hospital on that day with an erythrocyte count of 2.49 million, a hemoglobin of 7.6 gm. (48%), a white blood count of 7000, and a reticulocyte count of 0.4%. Sternal bone marrow obtained at that time showed megaloblastic arrest. A gastric analysis done after histamine stimulation showed the presence of free hydrochloric acid as well as rennin and pepsinogen in the gastric secretion. The oral glucose tolerance tests were again essentially normal. The icterus index was 14.1 units, and the urinalyses and stool analyses were essentially normal. By June 17, the red blood cell count was 1.60

million, hemoglobin 5.8 gm. and reticulocyte count 1.2%, and therapy was instituted. The patient received 7.5 gm. of synthetic 5-methyl uracil twice daily by mouth for a period of 9 days. A peak reticulocytosis of 31.2% was reached on the 7th day of therapy. The hematopoietic response of this patient is shown in Table 4 and in Figure 2. There was an improvement of appetite which lasted for a short period of time as well as an increase in well-being following the initiation of therapy. The symptoms of his glossitis and peripheral neuritis remained essentially unchanged. The highest red blood count attained was 3.13 million on the 21st day after the initiation of treatment. The hemoglobin at that time was 9.2 gm. By the 54th day after

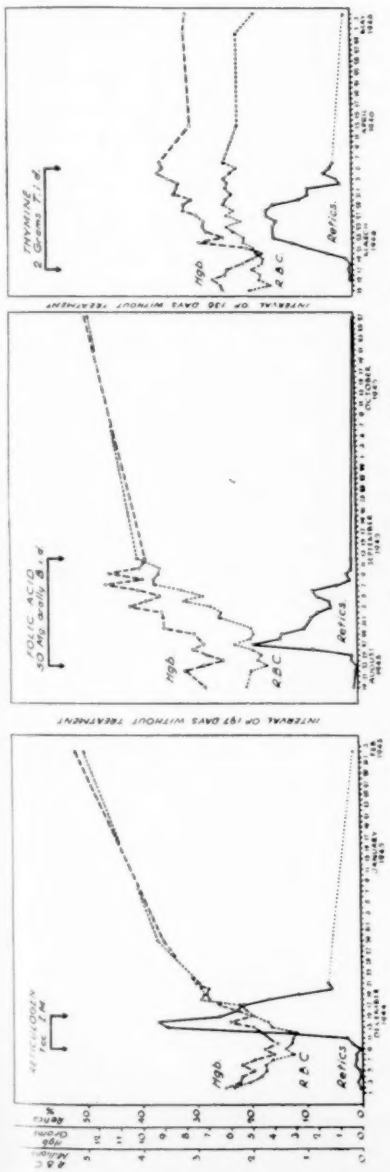

FIG. 1.—J. B., white male, age 46. Histamine refractory achlorhydria and achylia.

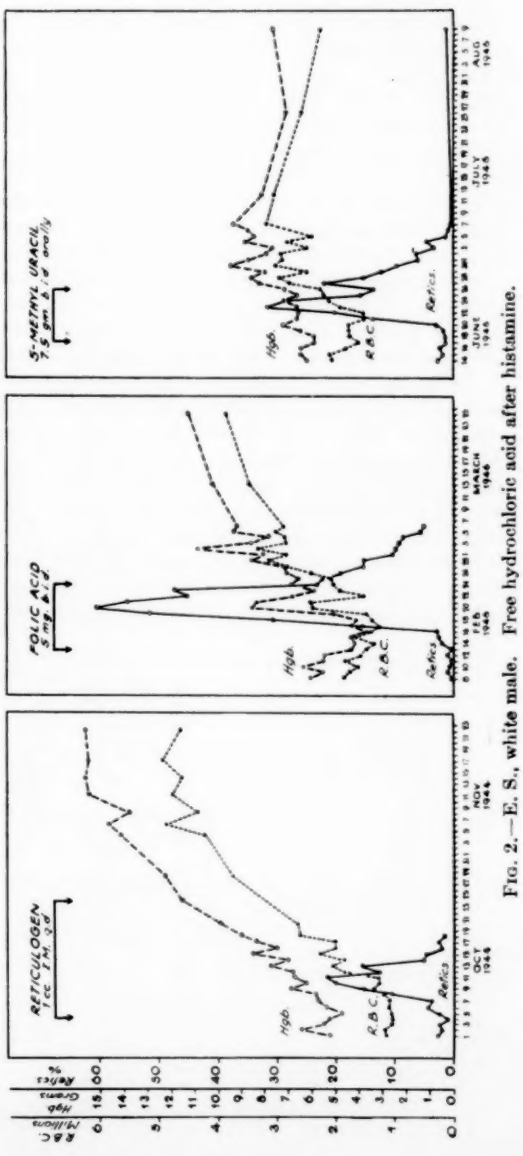

FIG. 2.—E. S., white male. Free hydrochloric acid after histamine.

therapy had been instituted, the erythrocyte count had fallen to 2.22 million and the hemoglobin to 7.5 gm. The patient had clinically and hematologically relapsed once more.

CASE 3. J. C. L. (tropical sprue). This 63 year old white male was admitted to the Calixto Garcia Hospital in Havana, Cuba, on Nov. 18, 1945, with complaints of weakness, vertigo and diarrhea. He dated the onset of his illness to 7 months prior to hospitalization. At that time he noticed burning and soreness of the tongue and the occurrence of small, tender ulcers in his mouth. At about the same time, he began to have from 10 to 12 bulky, frothy, foul-smelling, light-colored stools a day. Defecation was accompanied by colicky pain in the abdomen and by rectal burning. The patient lost his appetite, became weak, had shortness of breath, and lost 30 pounds in weight during the 7 months he was ill. His diet was evaluated as being deficient in animal protein, thiamine (B_1) and niacin. Physical examination revealed an emaciated, pallid male. His tongue was slick and red, and he had a fine, generalized, scaly desquamation of the skin over the entire body. Laboratory studies showed a flat oral glucose tolerance curve, free hydrochloric acid and enzymes in the gastric juice after histamine stimulation, and a normal urinalysis. The stools had a pH of 5.5 and were "fatty." Blood studies on admission showed a red blood cell count of 1.38 million and a hemoglobin of 6.4 gm. Baseline studies were completed by November 29, and on that date the erythrocyte count was 1.57, the hemoglobin 7 gm. (45%), and the reticulocyte count was 1.1%. The PCV was 21, and the blood indices were as follows: MCV 134, MCH 44.5 and MCHC 30. These blood indices as well as those of Case 4 are shown in Table 5. Therapy was instituted on that date, 10 mg. of synthetic folic acid being given daily by mouth for 82 days. Figure 3 and Table 6 show the hematologic response effected in this patient. A peak reticulocytosis of 27.2% was reached on the 8th day of therapy. Sternal bone marrow study at this time was normoblastic and in a reactive state. Within 10 days after treatment had been instituted, his stools became normal in color. They were copious and semisolid on some days and small and well formed on others. His appetite improved; his strength returned; he gained weight; and there was a striking improvement in his glossitis. On the 61st day after treatment was initiated, his red blood cell count was 3.96 million and his hemoglobin was 12.7 gm. By this time his stools had become almost normal. Eighty-nine days after therapy was started, the erythrocyte count was 4.88 million and the hemoglobin 13.3 gm. These were the highest levels obtained in this patient and represented a net increase in erythrocytes of 3.31 million and in hemoglobin of 6.3 gm. in a period of 89 days.

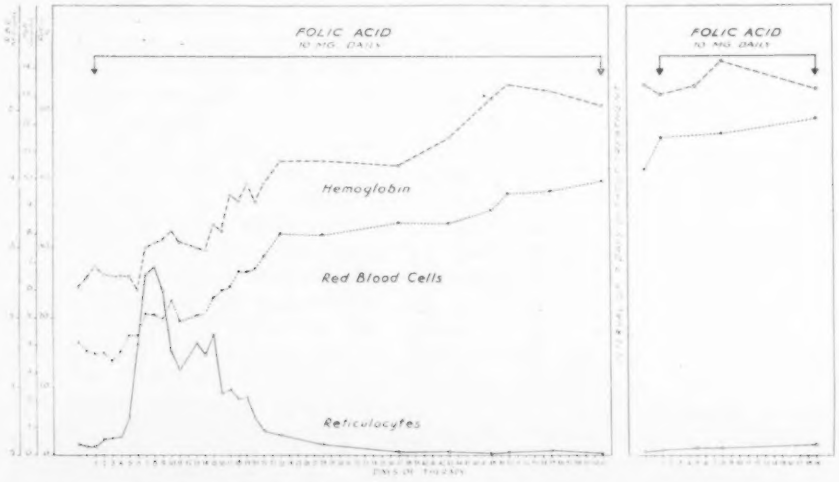

FIG. 3.—The effect of folic acid on the macrocytic anemia of tropical sprue.

TABLE 4.—HEMATOLOGIC RESPONSE OF CASE 2 (NUTRITIONAL MACROCYTIC ANEMIA) TO CONCENTRATED LIVER EXTRACT, SYNTHETIC FOLIC ACID AND SYNTHETIC 5-METHYL URACIL

Date of initiation of therapy	Erythrocytes (millions)			Hemoglobin (gm.)			Reticulocytes				Treatment and dose	No. days administered
	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial % (on 1st day of therapy)	First day of rise	Day of peak	% at peak		
10/4/44	1.03	2.05	5.95 (58)	4.2	6.2	12.7 (58)	1.0	3	8	21.4	Reticulogen, 1 cc. I.M.	15
2/13/46	1.46	1.80	3.81 (41)	4.3	6.8	11.2 (41)	0.4	5	8	60.4	Synthetic folic acid, 5 mg. b.i.d. orally	12
6/17/46	1.60	2.23	3.13 (21)	5.8	7.1	9.2 (21)	1.2	5	7	31.2	Synthetic 5-methyl uracil, 7.5 gm. b.i.d. orally	9

TABLE 5.—PACKED CELL VOLUME AND BLOOD INDICES OF CASE 3 AND CASE 4

Date	Case No.	PCV	MCV	MCH	MCHC	Color index
11/29/45	3	21	143	44.5	30	1.4
4/8/46	4	30	142	47.0	33	1.5

TABLE 6.—HEMATOLOGIC RESPONSES OF CASES 3 AND 4 (TROPICAL SPRUE) TO SYNTHETIC FOLIC ACID AND SYNTHETIC 5-METHYL URACIL

Date of initiation of therapy	Erythrocytes (millions)			Hemoglobin (gm.)			Reticulocytes				Treatment and dose	No. days administered
	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial (on 1st day of therapy)	10 days	Maximum (day)	Initial % (on 1st day of therapy)	First day of rise	Day of peak	% at peak		
						CASE 3						
11/29/43	1.57	2.23	4.88 (89)	7.0	8.1	13.3 (89)	1.1	5	8	27.2	Synthetic folic acid, 10 mg. daily by mouth	82
						CASE 4						
4/12/46	1.97	2.58	4.03 (63)	8.7	11.3	13.7 (63)	1.2	5	9	17.0	Synthetic 5-methyl uracil, 15 gm. daily for 14 days; 5 gm. daily for 125 days	139

CASE 4. A. H. (tropical sprue). This 37 year old white male was admitted to the Calixto Garcia Hospital in Havana on March 23, 1946, complaining of diarrhea and anorexia of 3 years duration. His illness began with severe meteorism followed by diarrhea consisting of 4 to 6 liquid or semiliquid, yellowish, foul-smelling stools a day. Defecation was accompanied by a sensation of rectal burning. Anorexia became severe; his mouth and tongue became sore; he developed paresthesias of the hands and legs, pain in the epigastrium, and profound weakness, all of which waxed and waned during that period of time. Four months prior to admission to the hospital, during a relapse, he received liver extract and some unknown medication for a period of $1\frac{1}{2}$ months from his private physician, and striking improvement followed this therapy. However, $2\frac{1}{2}$ months prior to admission he began losing weight, and within this period of time he lost 25 pounds. He relapsed rapidly 1 month prior to admission. The dietary history indicated that for many years his diet had been inadequate in all nutrients. Physical examination revealed a severely emaciated, pale man who appeared extremely depressed. Scaling of the skin was generalized. Over the arms, knees and shoulders there were areas of hyperpigmentation of the skin. The tongue was slick and edematous and was a brilliant red at the tip and edges. There were several blotchy red areas over the dorsum. The buccal mucous membrane also was red, but there were no ulcerations. Abdominal examination revealed a tympanitic percussion note but no other abnormalities. Neurologic examination was essentially negative. Laboratory studies revealed a flat oral glucose tolerance curve, the presence of free hydrochloric acid and enzymes in the gastric secretion after histamine stimulation, and a normal urinalysis. Stool examination showed a pH of 7.5 and an increase in total fat with a relative increase in fatty acids. Blood studies done on April 8, 1946, revealed a red cell count of 2.11 million, hemoglobin of 10 gm. (65%), a reticulocyte count of 1.2%, and a leukocyte count of 7700. The packed cell volume was 30, MCV 141, MCH 47, MCHC 33 and color index 1.5 as shown in Table 5. Sternal bone marrow showed typical megaloblastic arrest. By April 12 the red blood cell count was 1.97 million, the hemoglobin 8.7 gm., the reticulocyte count 1.2% and therapy was started. The patient was given 7.5 gm. of 5-methyl uracil by mouth twice daily for 14 days and then 5 gm. a day for 49 days. The initial reticulocyte rise occurred on the 5th day of therapy and reached a peak of 17% on the 9th day. The hematologic response is shown in Figure 4 and Table 6. Aspirated sternal bone marrow obtained on the day following the peak reticulocytosis

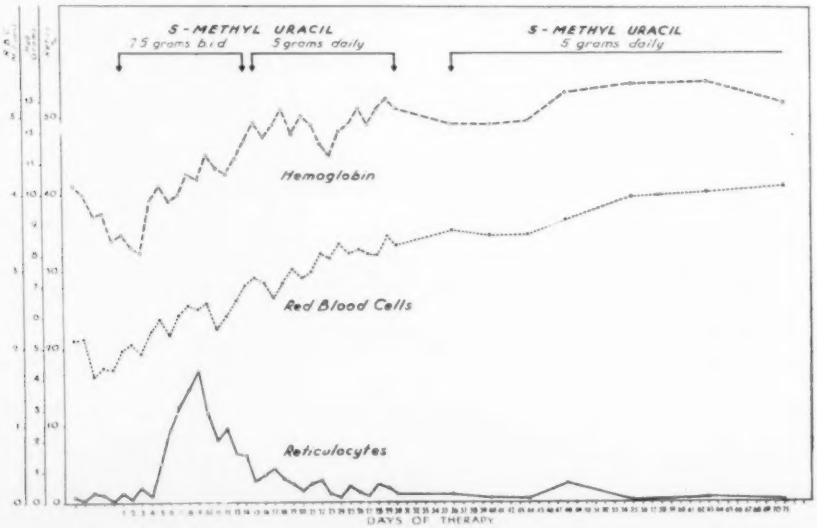

FIG. 4.—The effect of 5-methyl uracil on macrocytic anemia of tropical sprue.

was normoblastic and indistinguishable from the sternal bone marrow obtained from Case 3 at an analogous time during the latter's reticulocyte response to treatment with synthetic folic acid. After 8 days of treatment there was a striking increase in the patient's appetite. His tongue became normal subjectively, and there was temporary objective improvement; but both of these subsequently relapsed. His stools decreased to 1 a day, were dark brown in color, and tended to be normal in consistency although they were not well formed. By the 63rd day after treatment had been instituted, the red cell count was 4.03 million, and the hemoglobin was 13.7 gm. (89%). Therapy was maintained on this patient and a maximum erythrocyte count of 4.76 million was attained on the 139th day of treatment. The hemoglobin on that day was 12 gm. (78%). These figures are not included in Table 6 or in Figure 4. By this time the patient felt well and strong and had no subjective complaints.

Discussion. An analysis of Figure 1 brings out certain points of apparent difference in the hematologic responses of a patient with the macrocytic anemia of pernicious anemia in relapse who received concentrated liver extract, synthetic folic acid and synthetic 5-methyl uracil on 3 different occasions. At the time of administration of each of these substances, this patient's erythrocyte count was essentially the same. Because of this and because of the fact that his general condition at the time of each separate course of therapy was also essentially the same, it is reasonable to expect almost identical reticulocyte responses to each of these substances if they are equally hematologically effective. It is clearly evident that such identical responses were not obtained. The reticulocyte peak was highest with concentrated liver extract, next highest with folic acid, and lowest with 5-methyl uracil. Differences in rate of red cell rise are also evident. With concentrated liver extract as the therapeutic agent, there was an increase in erythrocytes of 3.69 million per c.mm. of peripheral blood in a 57 day period of time. This brought the red blood count of Case 1 to 5.08 million. When synthetic folic acid was used as the therapeutic agent, there was a red cell increase of 3.33 million in a similar period of time (65 days). This rise brought the patient's red cell count to 4.97 million. When synthetic 5-methyl uracil was employed as the only form of anti-anemic therapy, the erythrocyte level reached a peak of 2.39 million in 21 days. This represented a net increase in red cells of 530,000. By the 60th day after therapy had been instituted, the patient had already relapsed. An analysis of the 3 clinical responses attained in Case 1 to each of the substances employed in therapy is a far more difficult matter. Upsurge in well-being is almost indescribable; yet it is a very definite and important reaction. This upsurge in well-being occurred at about the same time in Case 1 when either concentrated liver extract or synthetic folic acid was the therapeutic agent. In effect there is little noticeable difference in the time of its occurrence or degree of its magnitude when either of these substances is used. There is a noticeable and very definite lag period in the time of onset in this sign of improvement when 15 gm. of synthetic 5-methyl uracil is the sole therapeutic agent given daily. Using this substance, we have been impressed by the fact that this reaction usually occurs some 3 to 5 days later than it does with either folic acid or liver extract. In Case 1, however, the manifestations of an upsurge of well-being never occurred when 5-methyl uracil was administered. The symptoms referable to glossitis are more consistently relieved by concentrated liver extract and folic acid than by 5-methyl uracil in the dose given. It has been observed that there has been no change in the glossitis in several of our patients treated with this latter substance. An increase in appetite and disappearance of weakness occurred with the same rate of speed in Case 1 when either liver extract or folic acid was administered. Initially this patient showed a similar response to 5-methyl uracil, but soon the response was

lost. The symptoms referable to paresthesias are much more often relieved with concentrated liver extract than with either folic acid or 5-methyl uracil. In general it can be said that, with the use of either sufficient concentrated liver extract or sufficient synthetic folic acid, there occurred an almost identical clinical response and subsequent improvement in Case 1 but that the height of the reticulocyte response and the rate of the red cell rise was of a slightly lower order following the administration of folic acid than it was following concentrated liver extract therapy. Synthetic 5-methyl uracil effected a less dramatic clinical and hematologic response than did either concentrated liver extract or folic acid.

Figure 2 represents the hematologic response of Case 2 (nutritional macrocytic anemia) to these same 3 substances. The reticulocyte response to folic acid was tremendous, but the rate of red cell rise was definitely slower than it was with the use of concentrated liver extract in dosage described. When liver was administered for 15 days, there was an increase in erythrocytes of 4.92 million over a period of 58 days. When folic acid was given, the increase in erythrocytes was only 2.35 million over a 41 day period; and a count of 4 million red blood cells was never reached. The reticulocyte response and the rate of red cell rise following 5-methyl uracil therapy were both of a lower order than those obtained by the use of either of the other 2 substances. The clinical responses of this patient to these 3 substances in the doses given were of the same order and magnitude as discussed under Case 1.

A comparison of Figures 3 and 4 shows a less dramatic hematologic response to synthetic 5-methyl uracil than to synthetic folic acid in persons with the macrocytic anemia of tropical sprue. The delayed clinical response following the administration of 5-methyl uracil is obvious to those who have used both substances. Folic acid in some fashion acts very much as does liver extract in that there is some indefinable general systemic effect which manifests itself as a generalized increase in strength or well-being or state of mind. Synthetic 5-methyl uracil does not have this function to the same degree at the dose level used by us.

The observed differences in the hematologic response to concentrated liver extract and synthetic folic acid are almost academic. The clinical response to folic acid is good, and it is almost identical with that of concentrated liver extract. However, for constancy and speed of reaction, concentrated liver extract is the more predictable of the 2 substances. The rate of red cell rise following folic acid therapy in these macrocytic anemias is somewhat slower than that which follows the administration of concentrated liver extract. Each substance has its advantage. Folic acid is easy to administer, and only small amounts by mouth are required for a very good response. Concentrated liver extract sometimes causes a more rapid increase in red cells. Synthetic 5-methyl uracil probably has no place in practical therapeutics. It has the distinct disadvantages of being effective only in very large amounts and of producing a clinical and hematologic response of a lower order than those effected by either concentrated liver extract or synthetic folic acid. Scientifically, however, 5-methyl uracil is of great importance. The authors are dubious that synthetic folic acid or synthetic 5-methyl uracil will prevent the development of the neural disturbances of Addisonian pernicious anemia.

These observations support the current idea that liver contains multiple antianemic substances. It is known that there is not enough folic acid in concentrated liver extract or crude liver extract to account for the activity of these various preparations. Obviously there is not enough 5-methyl uracil present. There still remains an unknown or a group of unknowns in liver which effect hemopoiesis. It is our opinion, and the opinion of others, that 5-methyl uracil may be converted into folic acid, possibly in the

intestinal tract, and in that manner cause maturation of red blood cells. This idea is not borne out by the substitution experiments of Stokes. How folic acid performs its function is as moot a question as that of how liver extract functions in such very small amounts. These are problems to be solved by the physiologists, biochemists and bacteriologists.

Summary and Conclusions. Comparative clinical and hematologic studies on the macrocytic anemia of pernicious anemia, of nutritional deficiency, and of tropical sprue, by the use of concentrated liver extract, synthetic folic acid, or synthetic 5-methyl uracil are presented. The points of difference in the clinical response, the reticulocytosis and rate of red cell rise following the administration of each of these 3 substances are discussed.

The hematologic findings show that under the conditions of the study and in the dosages used, each of these 3 substances causes a remission. The clinical and hematologic response to folic acid parallels that which occurs following concentrated liver extract therapy, but the rate of regeneration is greater with potent liver extract. We especially recommend folic acid where there is sensitivity to liver. Synthetic 5-methyl uracil is the least effective of the 3 substances. The rate of red cell rise and the peak attained is definitely of a lower order than that obtained with either synthetic folic acid or concentrated liver extract. While 5-methyl uracil is of great scientific interest, it has no practical therapeutic value at the dosage level required to maintain a normal red blood count. From these and other studies it seems to the authors that the active principle in liver extract is not folic acid but is a very powerful substance which, when obtained in pure form, probably will be no more efficacious per unit weight than is folic acid.

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CLINICAL SIGNIFICANCE OF HYPERBILIRUBINEMIA DUE TO NICOTINIC ACID

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and

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Villa[5] on the basis of extensive clinical trial advocates nicotamide in the treatment of various types of liver dysfunction. This drug appears to produce a striking remission of jaundice, provided the liver damage has not reached the irreversible stage.

The effect of nicotinic acid on the bile excreting function of the liver is the subject of the present investigation. The results obtained fully support Villa's conclusions.

As shown by Mattei,[4] 60 to 90 minutes after an intravenous injection of 30 mg. of sodium nicotinate, the level of the indirectly reacting serum bilirubin rises, remains high for 2 to 3 hours, and then drops slowly to normal in 6 to 8 hours (Fig. 1).

This technique is obviously valuable for investigating the function of the liver to excrete bile, since it acts as an endogenous bilirubin tolerance test. The cholemic effect of nicotinic acid seems to be specific, as several closely related substances (nicotamide, niketamide) as well as a number of compounds with similar pharmacological action (histamine, acetylcholine and epinephrine) appear to be inactive. Injections of histamine or epinephrine cause a change in the serum bilirubin level, but the rise is never higher than that observed during the normal diurnal fluctuation.

In studying the mechanism of the action of nicotinic acid, we also observed the behavior of serum chlorides, uric acid, erythrocytes and reticulocytes after the injection of this agent. Since these constituents were not significantly changed, it appears fairly certain that nicotinic acid does not mobilize bile pigments from tissue reserve, nor cause an increase of the normal hemolytic process. It is, however, possible that nicotinic acid activates normal oxidative processes, since it is known, for instance, that coproporphyrin, which is present in large amounts in pellagra blood (Frontali[1]), is strikingly diminished after nicotamide therapy. According to Kühnau,[2] nicotinic acid is important in the normal catabolism of blood pigments.

We have so far studied the bilirubin response to nicotinic acid in 42 patients with hepatic disease. These patients were also studied with other liver function tests following Watson's profile scheme.[6,3] The results with the nicotinic acid test clearly show that the capacity of the liver to excrete bile is measured. When this function is deficient, the serum bilirubin level is still high after 8 hours, and directly reacting bilirubin is present in the blood.

In non-jaundiced patients in which the bilirubin level is elevated, the bilirubin curve is normal, showing that the function to excrete bile is unimpaired even when hepatic dysfunction of varying degree is indicated by other function tests.

A higher response to nicotinic acid is observed in jaundiced patients whether caused by infective hepatitis, obstruction, arsenical poisoning, cholangitis, or other factors. This is probably due to two factors: (1) the mobilization of pigments from the tissues into the blood stream, and (2) the elimination of this excessive bilirubin in the blood depending on the efficiency of the excretory function of the liver.

In cases involving only the first factor, a marked rise of total and directly reacting bilirubin is observed, but after 8 hours the level is back to normal, due to the efficiency of the liver to excrete pigments. Several diseases of the liver of which the recovery stage of infective hepatitis is typical show this type of curve (Fig. 2).

to rise after the 8th hour (Fig. 4). Conditions in which this was observed were obstruction from new growth and from calculi as well as in the early icteric stage of infective hepatitis.

It is worth recording the paradoxical effect we observed in a case of jaundice due to poisoning from parenteral arsenic.

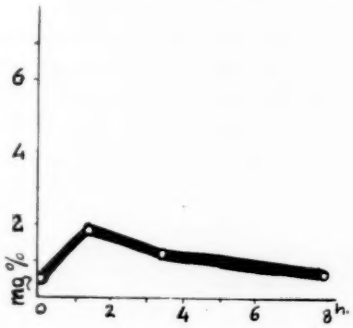

Fig. 1

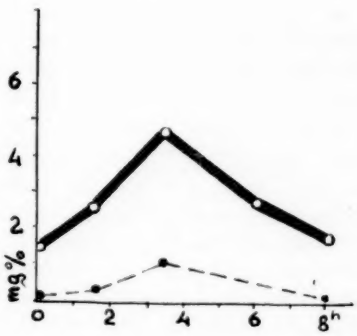

Fig. 2

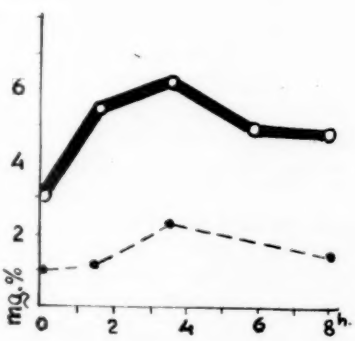

Fig. 3

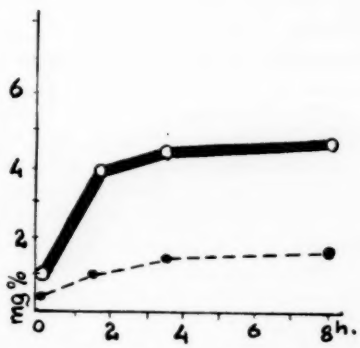

Fig. 4

Total serum bilirubin

Direct reacting bilirubin

In cases involving a disturbance of the biliary excretion function, the level of both the total and the direct reacting bilirubin rises and remains high even after 8 hours (Fig. 3). If complete blockage of the bile excretion exists, the total and the direct reacting bilirubin levels continue

The total and direct acting bilirubin remained unchanged for the first 60 to 90 minutes, then dropped slowly to much lower levels. The rapid excretion of bilirubin, possibly due to the stimulation by nicotinic acid, is a satisfactory explanation and suggests why nicotinic acid has pro-

phylactic and therapeutic activity in liver poisoning from drugs such as arsenobenzene.

Two of our observations clearly demonstrated that the behavior of serum bilirubin after nicotinic acid is strictly dependent on the capacity of the liver to excrete biliary pigments. In one patient suffering from jaundice of 5 months' duration due to chronic cholangitis, who underwent a cholecystostomy, the intravenous injection of nicotinic acid was followed by an increased excretion of bilirubin in the bile. It rose from 0.4 to 1.36 mg. per 100 cc. with the maximum at the third hour. In another patient, a case of infective hepatitis in the early stage, a curve of the type given in Fig. 4 was observed. After 5 days the test was repeated and found that the curve for bilirubin after nicotinic acid was normal which indicated that the biliary excretion of the liver was completely restored and suggests that this function is only temporarily depressed in infective hepatitis.

Summary. The serum bilirubin level was followed for 8 hours after the injection of 30 mg. of sodium nicotinate in several types of liver disease to demonstrate experimentally the therapeutic value of this agent. In a normal subject, nicotinic acid causes a rise of the indirect reacting bilirubin level for 3 to 5 hours. Such an action is not observed when other substances similar to nicotinic acid, either in structure or pharmacological action, are injected. The test is strictly dependent on the liver's capacity to excrete bile. Cholesterol and bile salts do not generally follow the variations of bilirubin. The reason for this is still uncertain and is being further investigated.

In 42 patients the bilirubin curve was found not to be typical for any particular type of liver disease, but depended on the capacity of the liver to excrete bile pigments. When this function was impaired the serum bilirubin remained high and direct reacting bilirubin appeared.

In a patient with long standing jaundice who underwent a cholecystostomy a direct stimulation of the liver's function to excrete bile was demonstrated.

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ATYPICAL ANEMIA, WITH SPHEROCYTES AND TARGET CELLS COËXISTING IN THE BLOOD

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Target cells, studied in detail by Barrett,[1] are very thin cells which in stained films have a deeply staining center so that they look like a bullseye; because of their thinness, the volume-thickness ratio is large, so that they can absorb large volumes of fluid from hypotonic solutions before they rupture, that is, they have properties opposite to those of the spherocyte, and in particular they show decreased fragility. They are most evident in sickle cell anemia and in Cooley's erythroblastic anemia of childhood[3] and its adult form,[8,20,22] all of which show decreased red cell fragility, lysis being inappreciable at salt concentrations greater than 0.35% NaCl. Such cells are, however, common in other conditions, notably obstructive jaundice and iron deficiency anemia of color index below 0.6, and there show their resistance to lysis by hypotonic solutions quite clearly.

This case is presented because target cells and spherocytes coëxisted in the blood, so that lysis of red cells was just appreciable in 0.60% NaCl, 50% complete in 0.335% NaCl, but incomplete in distilled water.

Case Report. A male office worker, aged 37, had always been in good health until in November 1944 he developed a cough and noticed that he was becoming pale and weak. He was admitted to Hertford County Hospital, where no disease was found in his lungs, but the liver was found palpable 2 inches below the costal margin. The spleen was not palpable. His hemoglobin was "55%" and "bizarre" changes were noted in the stained film. He remained in hospital for 6 weeks, receiving preparations of iron, vitamins and liver; during this period the hemoglobin rose to "71%," but this appeared unrelated to treatment. In June 1945 he was referred to Dr. Geoffrey Evans at Cell Barnes Hospital (E. M. S.) for further investigation.

There was no family history of anemia; his sister and his 2 children had normal hemoglobin levels and showed no abnormalities in the stained film. His parents and grandparents were not related, and were to the best of his knowledge, of English country stock (without any admixture of Levantine blood). He complained only of weakness and lassitude, with shortness of breath on exertion. He slept poorly. He was a well-built man with a sallow, subicteric complexion, and had received in childhood a fracture of the nose to which he, and ourselves, attributed a slightly mongoloid appearance. There was moderate pallor of the mucosa, gross dental caries and pyorrhea alveolaris. The lungs were clear, the heart normal except for a localized apical systolic bruit. The liver was palpable 1½ inches below the costal margin, but the spleen was not clinically enlarged. There was no enlargement of lymph nodes, no leg ulcers, and no changes in the skull or in the long bones could be detected.

Investigations. *Radiologic:* long bones, skull and alimentary tract were normal; the spleen was enlarged to about twice its normal size; chest normal.

Chemical: gastric analysis was normal; stools contained no occult blood; urine contained excess urobilin.

Gastroscopy (G. W.) showed only extreme pallor of the mucosa; *sigmoidoscopy* (Dr. Evans), no abnormality found.

Blood and marrow (June 26, 2945): Hemoglobin, 52% (7.4 gm. per 100 cc.); erythrocytes, 3,600,000 per c.mm.; hematocrit, 27.6%; color index, 0.73; M.C.V., 77 $c\mu$; M.C.H.C., 26.8%; M.C.H., 20.6 $\gamma\gamma$; halom-

eter reading, 8 μ; Price-Jones curve, mode 8 μ, mean 7.12 μ; standard deviation, 1.29 μ; coefficient of variation, 18.15%; M.C.T., 1.38 μ; fragility (linear on arithmetic probability paper), from 0.6% NaCl (0.27% lysis) to 0.16% NaCl (96.8% lysis); a few cells intact in distilled water; M.C.F., 0.355% NaCl; standard deviation, 0.0875% NaCl (normal M.C.F., 0.422 ± 0.0284%; standard deviation, 0.03025 ± 0.00354%).[10]

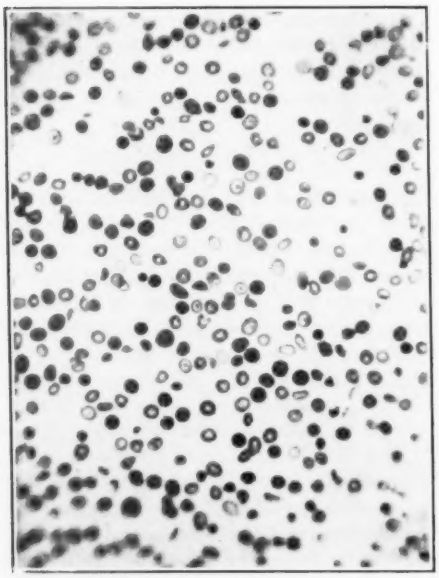

FIG. 1.—Peripheral blood, × 400; Leishman's stain.

Leukocytes, 5300 per c.mm.; immature myeloid cells, 5% (including a few myeloblasts); mature neutrophils, 32.5%; eosinophils, 9.5%; lymphocytes, 43.5%; monocytes, 5.5%; plasma and Türk cells, 1%. Nucleated red cells, with small pyknotic nuclei and scanty, ragged, orthochromatic cytoplasm, were present; 95 to 1000 leukocytes (about 500 per c.mm.). The erythrocytes showed extreme anisocytosis, with some very small but few very large cells, poikilocytosis and anisochromia; many pessary cells, target cells and spherocytes were present. Reticulocytes were scanty, never more than 1%; Howell-Jolly bodies were few and Cabot's rings were not seen. No sickling occurred in sealed preparations observed over a period of 48 hours. Grouping (Dr. Mourant): A1, Rh1, Rh2; Coomb's test[2,5,6] for adsorbed globulin negative.

Bilirubin: direct reaction, negative; total, 0.8 mg. per 100 cc.; Wassermann and Kahn tests negative.

Sternal marrow (Nomenclature Scott[21]): cellularity increased; leuko-erythrogenetic ratio, 0.227; erythropoiesis normoblastic, granulopoiesis shows many "P.A. giant stabs." Count: reticulum cells, 1.4%; myeloblast, 3.2%; promyelocytes, 2.6%; neutrophils: myelocytes, 7.8%; juveniles, 3.8%; stabs, 2.6% (of which half were giant forms); segmented, 2%; eosinophils: myelocytes, 2.4%; others, 0.6%; basophil myelocytes, 0.2%; lymphocytes, 1%; plasma cells, 0.8%; monocytes, 0.2%; normoblasts: pronormoblast, 6%; basophilic, 11.6%; polychromatic, 37.4%; orthochromatic, 19.6%; unclassified, 1%. Mitoses: granular cells, 0.4%; erythroblasts, 2.2%.

Cytologic abnormalities were numerous and prominent, and were studied in Leishman and Feulgen smear preparations; among the granulocyte precursors there was nucleic acid starvation comparable with that seen in pernicious anemia,[16] leading to the production of the tetraploid giant "stabs" once thought to be as characteristic for pernicious anemia as are the changes in the erythroblasts. Among the erythroblasts the outstanding feature was the breakdown of the normal spindle development and its replacement by abnormal multipolar spindles; of 100 mitoses, 55 were multipolar, 10 bipolar, and 35 unclassifiable because of clumping of the chromosomes. Many of the multipolar figures showed incomplete separation at anaphase, with persistent bridging leading to the restitution of a single, large (up to 16 μ) distorted, hyperploid, resting nucleus; frequently micro-nuclei, derived from chromosomes which had become detached from the spindle, were also present, and a few cells containing up to 20 micro-nuclei were found. These last are presumably the product of a cell division in which many chromosomes became dissociated from the spindle and failed to constitute a daughter-nucleus. In addition, hypoploid mitoses were present, an occasional cell showing as few as a dozen chromosomes in a tri-polar mitosis. Cell division occurred mainly in cells corresponding to the "normoblast B" of Israels,[15] well differentiated, with much heterochromatin,

and with polychromatic or orthochromatic cytoplasm.

PROGRESS. No definite diagnosis was made. The patient received iron (ferrous sulphate), parenteral liver extracts of known potency (Anahæmin, Plexan), and proteolyzed liver by mouth (Hepamino) and vitamin supplements, and his hemoglobin rose again to 71%. Col. A. M. Washburn, M.C., A.U.S., kindly saw the patient with us, but was equally puzzled. Splenectomy was considered, in view of the evidence of increased hemolysis and of spherocytosis, but was postponed, and the patient discharged to remain under observation. On September 12 he was readmitted; during the previous week he had become much worse and was dyspneic on the slightest exertion.

Studies now showed: Blood: hemoglobin, 38% (5.4 gm. per 100 cc.); erythrocytes, 2,500,000 per c.mm.; hematocrit, 21.5%; color index, 0.76; M.C.V., 86 $c\mu$; M.C.H.C., 25%; leukocytes, 7400 per c.mm.; stained film, differential count and bilirubin showed little change.

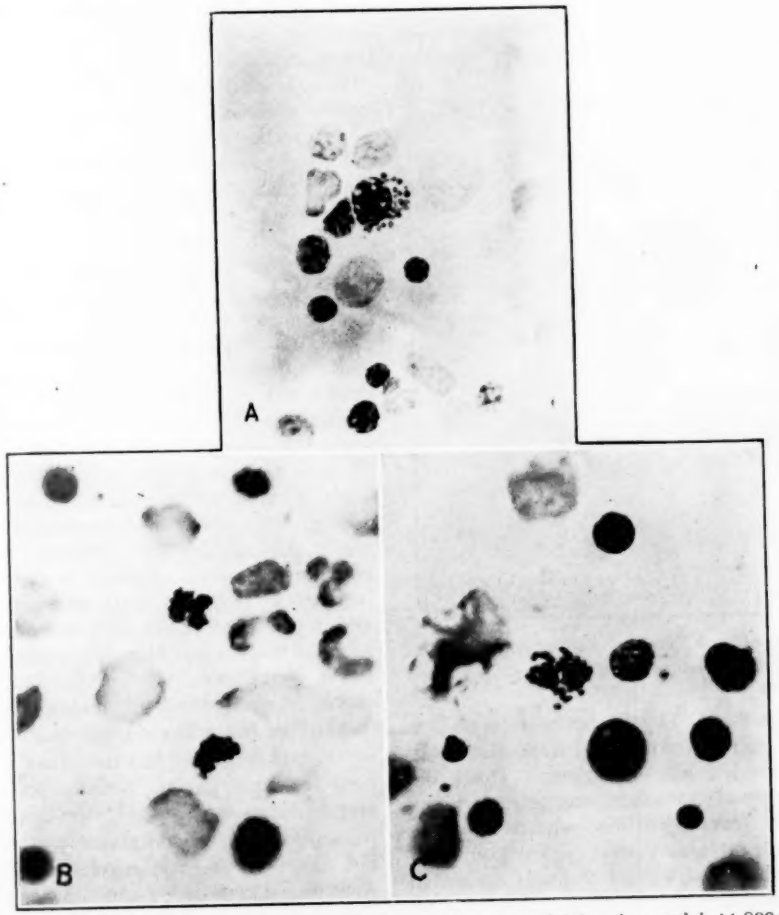

FIG. 2.—Erythroblasts in bone marrow; Feulgen stain: *A*, multiple micronuclei, × 800; *B*, anaphase bridge and multipolar spindle, × 950; *C*, breakdown of spindle, × 950.

He did not improve after a weeks rest, and the rise in hemoglobin after a transfusion of 500 cc. blood was evanescent; he developed fever up to 99°, and purpuric spots appeared on his trunk. It was decided to perform splenectomy before his condition became critical. The operation was per-

formed by Mr. J. P. Hosford on October 2, after transfusion of 1000 cc. packed red cells, which raised the hemoglobin to 70%; a blood film taken during anesthesia showed about 1 mature normoblast to every 50 red corpuscles, or about 70,000 per c.mm. The spleen, enlarged to twice the normal size, and a splenunculus about 1.5 cm. diameter, were removed, but 8 hours later, after recovery from the anesthetic, the patient collapsed with a feeble pulse (rate 120), and signs of collapse of the right lower lobe; he never recovered and died 4 hours later.

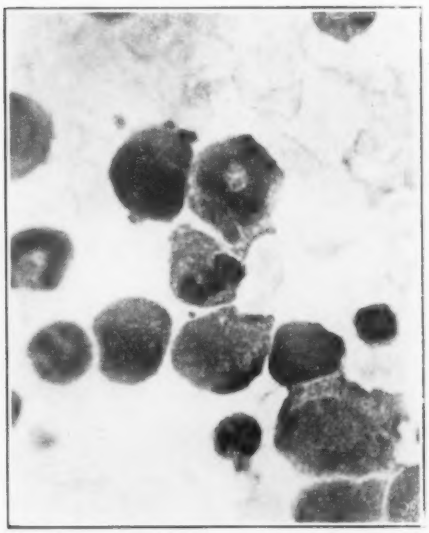

FIG. 3.—Giant "stab" neutrophils, × 800; Feulgen light green.

AUTOPSY (G. D.) 12 hours after death: External: rigor mortis and hypostasis present; left-sided Kocher incision. Head and brain normal; mouth, gums retracted and infected, several carious stumps; tongue, thyroid, normal. Chest, pericardium contains 50 cc. straw-colored fluid; epicardial fat increased, ventricles dilated, myocardium fatty and friable, small subendocardial hemorrhages in wall of left ventricle; great vessels normal. Respiratory organs: diffuse tracheobronchitis with submucous hemorrhages, increasing in severity in the smaller bronchi; erosion of mucosa at end of endotracheal tube: patchy collapse of the lungs, involving whole right lung and left lower lobe, collapsed regions intensely engorged and edematous. Alimentary system normal. Operation site: about 50 cc. blood as a firm clot over sutures, otherwise no blood in peritoneal cavity. Liver, 3 pounds 12 ounces, pale. Suprarenals normal. Kidneys, left 7 ounces, right 6 ounces, cortex pale, otherwise normal. Femur, vertebræ, ribs, skull, all contain red marrow.

HISTOLOGY. Trachea: mucosal atrophy with hyperemia of submucosal vessels, some epithelial desquamation. Lungs: all vessels engorged; patchy collapse and compensatory emphysema and edema; the bronchioles contain a cellular mononuclear exudate and there is sloughing of their epithelium; in some areas an acellular exudate appeared to close the alveoli as in "rheumatic pneumonia." Heart: myocardium shows slight fatty change. Liver: definite increase in free iron, confined to the liver cells; bile pigment increased in the center of the lobules; the sinusoids are dilated and contain many erythroblasts, and the portal tracts are infiltrated with erythroblasts; definite myeloid metaplasia. Kidney: recent cortical necrosis; in the pelvic fat there is a focus with the structure of bone marrow, though no bone is present; the predominating cell is an erythroblast; there is no excess of free iron. Pancreas and testis, normal. Bone marrow: hyperplastic, the predominating cell being an immature polychromatic normoblast, but granular cells are common. Spleen: there is a considerable increase in free iron, both in macrophages and in the tissue of the pulp, and an increase in the fibrous tissue; the pulp is packed with blood containing many erythroblasts, and there is some myeloid metaplasia, most marked in the splenunculus; there is some erythrophagocytosis, but this is not a prominent feature. Lymph nodes: the general architecture is preserved; the sinuses are packed with blood containing erythroblasts, but there is no true myeloid metaplasia. Anatomic diagnosis: pneumonia (virus type), cortical necrosis of the kidney, hyperplasia of bone marrow with myeloid metaplasia in liver, spleen and renal pelvic fat.

Discussion. The anatomic changes in this patient are not specific, but are common to many types of anemia in man, and to some syndromes in animals; it differs from pernicious anemia only in the normoblastic type of erythropoiesis and

in the focus of erythrogenesis in the renal pelvic fat. For this reason, study of the peripheral blood and of the cytogenesis of the red cell is more likely to prove fruitful than is the classic histologic approach; it is obvious that details of cell structure can be studied more satisfactorily in smears of cells dissociated from their surroundings and suitably treated, rather than in sections, where the cell retains the roughly spherical shape it had when living.

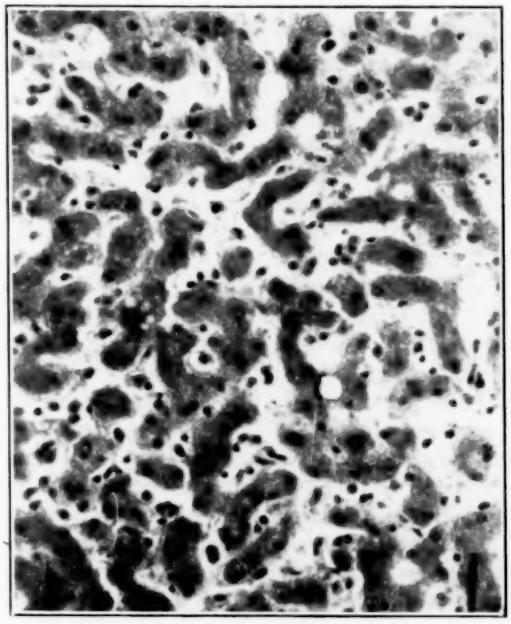

FIG. 4.—Liver, × 300; Barratt's stain.

FIG. 5.—Renal pelvic fat, × 150; Barratt's stain.

CYTOLOGY. The changes in this patient are not unlike those described in pernicious anemia by La Cour,[16] but differ in that cell division is proceeding in a much more mature cell; in pernicious anemia division occurs chiefly at the promegaloblast and megaloblast "A" stage, and is comparatively rare in more mature cells; a much larger proportion of the multipolar mitoses of pernicious anemia appear to yield good, though hypoploid, cells. There can be no doubt that this patient suffered from some condition which was not pernicious anemia, for the resting nuclei differed markedly from those of any pernicious anemia marrow yet seen, whether in relapse or in remission: it was normoblastic, not megaloblastic, using the terms in the same way as Ferrata,[12] and Israels,[15] but the normoblasts were abnormal. Several cases of hemolytic anemia, congenital and acquired, have been examined critically, but these show only very rare multipolar spindles, or anaphase bridging. The essential lesion appears to be the breakdown of spindle formation, yielding hyper- and hypoploid cells which undergo denucleation to yield red corpuscles that vary extremely not only in diameter but also in thickness.

The presence of micronuclei in this case led one to compare them with the Howell-Jolly bodies so common in pernicious and hemolytic anemia. Both are Feulgen positive, and hence composed largely of deoxyribose nucleic acid; they are sharply defined, unlike the nucleic acid shed from the nucleus under the influence of irradiation or of cytotoxic agents, and so presumably have some inner structure. In material in which Howell-Jolly bodies are common in the peripheral blood, the bone marrow shows similar bodies in the cytoplasm of nucleated red cells, while the mitotic figures of these cells show occasional telophases in which a single chromosome remains between the 2 groups of chromosomes, either exactly midway between, or else towards the margin of the cell, well outside the region of the spindle. One is forced to the conclusion that these bodies are not "nuclear remnants," but chromosomes dissociated from the spindle. In most cases this occurs in an almost mature cell, in which spiralization of the chromosome fiber (chromonema) is complete or excessive, so that the chromosomes are almost spherical and homogeneous; in less mature cells the chromonema is more extended and the resulting body may appear heterogeneous.

CLINICAL. *Differential Diagnosis.* Initially this patient was thought to be suffering from familial acholuric jaundice which had become clinically evident during adult life. Such cases are not rare, and the absence of clinical splenomegaly is not significant, for we have seen 3 patients with grossly enlarged spleens (1 with myeloid leukemia) which could not be detected at the bedside; this occurs when the spleen is adherent to, or pressed up against, the cupola of the diaphragm.[4] The spherocytosis favored this diagnosis, though it is not, of course, pathognomonic of the condition,[7] but the absence of reticulocytosis and the extremely wide range of fragility rendered the diagnosis untenable. For similar reasons the ordinary acquired hemolytic anemias could be excluded, for erythroblastemia of significant degree occurs only during, or just after a hemolytic crisis. Target cells were reported as a prominent feature in 1 case,[19] but this showed reticulocytosis and atypical agglutinins in the serum.

The adult form of Cooley's Mediterranean anemia is familial, and occurs almost exclusively in persons of Levantine origin, especially in the offspring of cousin marriages. In favor of this diagnosis were the presence of target cells and erythroblasts, hepato- and splenomegaly; but the patient was of pure English origin, his parents were not related, and none of the 3 siblings was affected; while spherocytes do not occur in this. This diagnosis also was discarded.

A leuko-erythroblastic reaction to marrow deposits of carcinoma, lymphadenoma, or to tuberculosis of the spleen could at once be discarded, as could the erythro-

blastemia of chronic malaria, stated[17] to be particularly common in Algeria; in this infection splenomegaly is considerable, and also our patient had never left England.

The condition bears little resemblance to the acute erythremia of Di Guglielmo,[9] but differs comparatively little on the clinical side from the 1 case of Israels[14] which was less acute. It is not unlike the erythroblastosis of fowls,[11] which can manifest itself in all grades of severity.

One must conclude either that this is a new syndrome, and that further cases will be recognized, or that it is an "individual" response to some stimulus, comparable with the "acute erythromyelosis" of Garnier,[13] with 35,000 erythroblasts per c.mm., 6% of which were in mitosis, which recovered in a month on treatment with liver extract. The only excuse for presenting single cases is that others may profit by our observations, define the syndrome and develop a satisfactory treatment.

One final point: usually erythroblastemia is associated with reticulocytosis, though the 2 responses do not necessarily run parallel. Pittaluga,[18] however, insists that "regenerative reticulocytosis has nothing to do with pathologic erythroblastosis," a rather surprising conclusion with which we are forced to agree.

Summary. 1. A patient suffering from an obscure anemia which failed to respond to treatment and terminated fatally after splenectomy is described.

2. This anemia is characterized by: (*a*) great variation in the resistance of the red corpuscles to lysis by hypotonic saline, manifested morphologically by the coexistence of target cells and spherocytes; and (*b*) breakdown of spindle development in partly differentiated erythroblasts.

3. This appears to be a hitherto undescribed syndrome.

We wish to thank Dr. Geoffrey Evans for permission to publish this case, and Mr. L. F. La Cour of the John Innes Horticultural Institute, who has advised us on the interpretation of the cytologic abnormalities and taken some of the photographs.

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NITROGEN BALANCE AND BLOOD VOLUME STUDIES IN MAN DURING AND FOLLOWING REPEATED PLASMA TRANSFUSIONS*

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Because of the wide use of plasma in the treatment of shock, and in certain cases of hypoproteinemia, there has been an increased interest in the fate or utilization of the plasma proteins. Elman[11] reviewed the literature up to 1943 and concluded that nitrogen balance can be maintained with plasma as the sole source of protein, providing the plasma employed causes no serious reactions. There is some controversy on this point, however, as the results of the nitrogen balance studies following intravenous infusion of plasma as reported by different laboratories have been at variance. It does not seem that these differences can be entirely explained by reactions to the injected plasma. Most of this work has been carried out on dogs which have received injections of dog plasma. Hegsted, Hay and Stare[17] have found through growth studies in rats that human albumin is deficient in isoleucine and tryptophane. However, they demonstrated that oral feeding of this protein maintained nitrogen balance in adult dogs. These species differences in the requirements of specific amino acids make it difficult to reach any satisfactory conclusion on the fate of human plasma injected in human beings from animal experiments.

Excluding conditions of shock, or the first several weeks following a burn or fracture, when nitrogen excretion is known to be increased above the normal,[18,20] there seem to be only 3 reports on nitrogen balance when human plasma was transfused into apparently normal individuals. Kreman, Hall, Koschnitzke, Stevens and Wangensteen,[21] and Wangensteen, Hall, Kremen and Stevens[32] compared the use of human plasma with bovine plasma, and Muether and Andrews[25] reported some studies on 6 patients in conjunction with methods of preserving blood. None of these studies in humans included the alterations in plasma volume or hematocrit which resulted from the transfusion. Although there is information on such changes immediately, or a few days following a single transfusion,[15,16,28] there are no reports on such alterations in the late post-transfusion period following repeated injections of plasma. The following study was, therefore, undertaken to obtain more information on the effect of human plasma transfusions on the nitrogen balance in man, and on changes in plasma volume, plasma proteins and hematocrit.

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Procedure. Three patients were selected for this study who seemed to be in a good state of nutrition.* Their case histories and detailed dietary management are given in the protocol. The following synthetic diet was fed in amounts to insure slightly greater than an adequate protein and caloric intake: Hydrolyzed casein (Amigen) dextromaltose and orange juice supplemented with vitamins. Iron was contained in the diet in amounts almost equal to the accepted daily requirements (15 mg.), and additional amounts were given in the form of iron citrate in the last 2 subjects.

The patients received the diet for at least 4 days, and then nitrogen balance studies were carried out for two 4 day periods on this diet. Following this, protein was omitted from the diet for 5 days and in its place the patient was given a daily intravenous injection of plasma estimated to contain the same amount of protein as he had eaten on each of the previous days. (Amount for each patient given in the protocol.) Subsequently the patients were returned to their control synthetic diet and studied for from two to four 4 day periods. In the last 2 subjects, following the completion of 4 periods after the transfusion period, another 5 day period was added in which the protein in the diet was entirely eliminated in order to study the nitrogen excretion and partition under these conditions. This period was essentially the same as the third period when plasma was administered except that the patient was not given any parenteral protein.

Food, feces and urine were analyzed for total nitrogen content, and the following nitrogen constituents of the urine were determined: urea, ammonia, uric acid, creatinine, creatine and amino acids. The methods for these determinations have been described elsewhere.[33] Plasma volume, hematocrit, plasma protein and albumin determinations were made during the control period, and 18 to 20 hours following the last transfusion. In the first subject these same studies were also made on the 9th day after the last transfusion; in the second subject, on the 12th and 23rd day, and in the third subject, on the 9th and 24th day. The method for these determinations was essentially the same as that previously employed.[1] Ten cc. of Evans blue dye (T-1824) containing 1 mg. per cc. were injected intravenously for the determination of the plasma volume. The total red cell mass was calculated from the plasma volume and hematocrit and while this method does not give reliable values,[14] it was felt that the percentage changes in the total red cell mass might be of some significance.

Results. The data on nitrogen balance and urinary nitrogen partition are summarized in Tables 1 and 2. All 3 subjects were in positive nitrogen balance during the first 2 periods while receiving the hydrolyzed protein by mouth, except for Subject C. S., who showed an insignificant loss during the first period. This confirms the reports[6,30] that hydrolyzed protein given orally will maintain nitrogen balance. During the 5 day period of plasma transfusion, all subjects were not only in positive balance, but the nitrogen retention during this period (particularly in Subjects J. M. and C. S.) was much greater than when nitrogen was fed in the form of casein hydrolysate. However, following the transfusion, the excretion of urinary nitrogen was increased over the control periods in all 3 subjects, although the retained nitrogen was not entirely lost. In the first subject, J. M., this resulted in only a slight loss, a total of 3.12 gm. in 8 days, or 13% of the amount gained (24.35 gm.) during the period of transfusion. However, in C. S., this loss was more marked, totalling 26.13 gm. in 9 days, or 85% of the amount gained (30.70 gm.). Subject C. M. lost a total of 7.68 gm. in 5 days, or 52% of the amount gained (14.90 gm.) during the transfusion period.

The urinary nitrogen excretion in the last 2 subjects on the protein-free diet was similar to that observed during the period of transfusion. The average nitrogen excretion in Subjects C. S. and C. M. during the periods of transfusion was 5 and 5.26 gm. respectively, whereas on the nitrogen-free diet it was 4.29 and 4.84 gm.

* Because of variation in age, nutritional state, etc., these cases were admittedly not ideal, but under the circumstances were the best that could be obtained.

TABLE 1.—DATA ON NITROGEN BALANCE

Case	Period	N intake: Amigen (gm.)	N intake: Orange juice (gm.)	N output: Urine (gm.)	N output: Feces (gm.)	N output: Emesis (gm.)	Nitrogen balance (gm.)	Body weight (kg.)
J. M.	1	13.43	0.37	12.50	0.61	..	0.69	62.0
	2	..	..	(11.42)				
				(11.85)				
				(11.26)				
				(12.19)				
		13.43	0.38	Av. 11.68	1.12	..	1.01	
	3	..	..	(8.54)				
				(6.21)				
				(5.31)				
				(6.53)				
				(5.25)				
		11.65*	0.49	Av. 6.37	0.77	0.13	4.87	
	4	..	..	(12.82)				
				(13.02)				
				(14.15)				
				(14.36)				
		13.43	0.48	Av. 13.59	0.75	0.04	−0.47	64.4
	5	13.43	0.44	13.53	0.65	..	−0.31	63.1
C. S.	1	9.74	0.26	9.10	1.18	..	−0.28	59.8
	2	9.74	0.29	8.88	0.78	..	0.37	59.8
	3	..	..	(6.93)				
				(4.21)				
				(4.59)				
				(4.84)				
				(4.43)				
		11.70*	0.31	Av. 5.00	0.87	..	6.14	59.3
	4	..	..	(9.00)				
				(14.29)				
				(13.67)				
				(12.75)				
		7.96†	0.21	Av. 12.43	0.89	0.02	−5.17	59.5
	5	9.95	0.22	10.70	0.56	..	−1.09	60.8
	6	9.74	0.27	8.97	0.78	..	0.26	60.0
	7	9.74	0.25	8.91	0.31	..	0.77	
	8	9.74	0.25	9.35	0.56	..	0.08	60.0
	9	..	..	(9.14)				
				(4.95)				
				(3.64)				
				(3.45)				
				(3.03)				
			0.24	Av. 4.84	0.47	..	−5.07	59.4
C. M.	1	11.30	0.55	8.43	1.28	0.01	2.13	67.1
	2			(10.26)				
				(8.92)				
				(7.48)				
				(8.20)				
				(8.31)				
		11.30	0.50	Av. 8.63	0.57	0.14	2.46	67.2
	3	..	..	(5.93)				
				(4.51)				
				(4.27)				
				(5.58)				
				(6.38)				
		8.13*	1.07	Av. 5.33	0.77	0.12	2.98	
	4	..	..	(10.72)				
				(15.43)				
				(14.22)				
				(12.93)				
		11.30	0.46	Av. 13.33	0.29	0.06	−1.92	
	5	11.30	0.43	10.31	0.80	0.01	0.61	
	6	11.30	0.47	8.66	1.05	0.30	1.76	
	7	11.30	0.46	8.27	0.78‡	..	2.71	
	8	..	0.44	4.29	0.68	..	−4.53	

* Nitrogen by plasma transfusion.

† Through error amigen omitted 1st day.

‡ Average fecal nitrogen value.

During the period of transfusion and the nitrogen-free diet these 2 subjects showed an increase in % uric acid, creatinine and creatine excretion. Similar alterations were observed in the first subject during the transfusion period. However, the absolute amount of these various fractions was relatively constant, except for creatine excretion which was markedly increased in the first subject (J. M.) during the period of transfusion and in the last subject (C. M.) in the period following transfusion. The % urea nitrogen excretion was lower in this patient during transfusion and on the nitrogen-free diet. It was also lower on the nitrogen-free diet (16%) but the value increased by the 12th day (24%) and returned to normal on the 23rd day. Subject C. M. showed no significant change on the 1st day following the last transfusion, but as in Subject C. S. showed an increase (16%) on the 12th day; on the 23rd day the value was 10% above the control.

Hematocrit values were decreased during and following the period of injection. In the first subject, J. M., the fall in the hematocrit appears to be largely the result of dilution rather than decrease in the volume of circulating red cells. But in the last 2 cases (C. S. and C. M.) the drop in hematocrit was marked and cannot be

TABLE 2.—URINARY NITROGEN PARTITION

Case	Period	Urea		Ammonia		Uric acid		Creatine		Creatinine		Amino acid'		Partition nitrogen % of Kjeldahl nitrogen
		Mg.	%	Mg.	%	Mg.	%	Mg.	%	Mg.	%	Mg.	%	
J. M.	2	9,722	83.2	476	4.1	180	1.5	13	0.1	425	3.6	788	6.7	99.2
	3	5,115	80.2	152	2.4	179	2.8	178	2.8	422	6.6	456	7.2	102.0
	4	11,306	83.2	475	3.5	250	1.8	92	0.7	422	3.1	953	7.0	99.3
	5	11,223	82.9	613	4.5	236	1.7	21	0.2	415	3.1	820	6.1	98.5
C. S.	1	7,257	79.7	504	5.5	196	2.2	14	0.2	334	3.7	418	4.6	95.9
	2	7,584	85.3	414	4.7	190	2.1	39	0.4	338	3.8	374	4.2	100.5
	3	4,233	85.0	196	3.9	142	2.8	35	0.7	321	6.4	275	5.5	104.3
	4	11,362	91.4	302	2.4	193	1.6	60	0.5	334	2.7	522	4.2	102.8
	5	9,014	84.2	450	4.2	210	2.0	55	0.5	337	3.1			
	6	7,595	84.6	459	5.1	200	2.2	55	0.6	340	3.8	452	5.0	101.3
	7	7,740	86.9	348	3.9	211	2.4	43	0.5	331	3.7	492	5.5	102.9
	8	8,236	88.0	307	3.3	146	1.6	50	0.5	323	3.4	426	4.6	101.4
	9	3,849	79.4	280	5.8	175	4.0	33	0.7	481	9.9	235	4.8	104.6
C. M.	1	6,742	80.0	524	6.2	130	1.5	36	0.4	622	7.4	523	6.2	101.7
	2	7,077	82.0	306	3.5	161	1.9	65	0.8	602	7.0	605	7.0	102.2
	3	4,139	77.6	213	4.0	199	3.7	77	1.4	529	9.9	499	9.3	105.9
	4	11,215	84.1	464	3.5	206	1.5	142	1.0	584	4.4	823	6.2	100.7
	5	8,840	85.7	598	5.8	184	1.8	67	0.6	483	4.7	619	6.0	104.6
	6	7,280	84.0	365	4.2	204	2.4	8	0.1	511	5.9	595	6.9	103.5
	7	6,596	79.8	436	5.3	179	2.2	44	0.5	565	6.8	793	9.6	104.2
	8	2,646	61.7	340	7.9	138	3.2	54	1.2	558	13.0	426	9.9	96.9

in the second subject (C. S.) but not during the period of transfusion. The decrease in % urea nitrogen excretion in the first subject (J. M.) during the transfusion seems insignificant. Ammonia and amino acid nitrogen excretion showed no marked alterations during the study.

As shown in Table 3, the changes in plasma volume following the transfusion were not consistent, but the trend was towards an increase. The first subject, J. M., showed a significant rise (38%) on the 1st day following the 5 days of transfusion, and the value was 11% above the control on the 9th day. The second patient (C. S.) showed only a small increase accounted for by dilution. On the 12th day the calculated volume of circulating red cells was 31 and 36% respectively below the control value in these 2 subjects. It was at least 23 days before the hematocrit and volume of circulating red cells approached the control value.

Protein concentration in the first subject showed only slight changes during the entire experiment, but Subjects C. S. and C. M. both showed an increase in the plasma protein concentration following the periods of transfusion. Alterations in albumin-globulin ratios were small, except in C. M. where the ratio was consistently higher than the control value.

Total circulating protein immediately following the transfusion was increased (33 and 52%) above the control value, but as shown in Table 4 the increase was small compared to the amount of protein injected during the 5 days.

average of 5.56% protein. The transfusion was given in 2 divided doses, morning and evening. The diet during this time contributed 2076 Cal.

The temperature of the patient was normal previous to the transfusion. He had a chill on the 1st day and the transfusion had

TABLE 3.—PLASMA VOLUME, PROTEIN AND HEMATOCRIT CHANGES

Case	Day of transfusion	Days following last transfusion	Plasma volume change (%)	Hematocrit change (%)	Blood volume change (%)	Circulating red cell mass change (%)	Protein concentration	Total circulating protein change (%)	A/G ratio
J. M.		(Control)	(3171)	(43.4)	(5602)	(2431)	7.42	(235)	1.32
	4			−10			7.96		1.46
		1	+38	−19	+21	− 2	7.16	+34	1.26
		3	..	−15	..	..	7.40	..	1.34
		8	..	−13					
		9	+11	−11	+ 3	− 8	6.79	+ 2	1.52
C. S.		(Control)	(2970)	(40.4)	(4983)	(2013)	6.37	(189)	1.47
	3			− 7	..	..	7.66	..	1.52
		1	+16	−29	− 3	−31	8.34	+52	
		4	..	−39	..	..			
		5	..	−46	..	..	8.52	..	1.16
		12	+24	−32	+ 2	−31	..	+38	1.41
		23	+ 4	−16	− 6	−21	6.46	+ 5	2.00
		30	..	+ 9	..	..	6.83	..	1.73
C. M.	..	(Control)	(2902)	(46.9)	(5465)	(2563)	6.72	(195)	1.43
		1	+ 4	−12	− 6	−18	8.78	+36	2.24
		9	+16	−30	− 9	−36	7.09	+22	2.12
		14	..	−17					
		24	+10	− 9	+ 2	− 8	6.55	+ 7	2.66

TABLE 4.—DIFFERENCE BETWEEN PROTEIN INJECTED AND INCREASE IN PLASMA PROTEIN

	Total circulating proteins				
	Before transfusion (gm.)	After transfusion* (gm.)	Increase (gm.)	Protein injected (gm.)	Difference between protein injected and increase in circulating protein (%)
J. M.	235	314	79	364	22
C. S.	189	287	98	366	27
C. M.	195	265	70	254	28

* 18 to 24 hours after last transfusion.

Case Histories. CASE 1. J. M., 65 year old white man, admitted to the hospital because of bilateral hernia. Right herniorraphy done on April 11, 1944, and left on April 25. Convalescence from operations entirely uneventful.

On May 5, patient was started on diet consisting of 109.2 gm. hydrolyzed protein, 473 gm. dextro-maltose, 500 cc. orange juice, 2 gm. salt mixture, 100 mg. ascorbic acid, 20 mg. thiamin, 50 mg. nicotinic acid, percomorphum 4 minims, made up in 1000 cc. of water. This diet provided 86 gm. of protein and 2440 Cal. The first collection period was started on May 10. During the 3rd period the patient received the following amounts of plasma on 5 successive days: 1340, 1100, 1305, 1480 and 1320 cc. The plasma was obtained from the Receiving Hospital Blood Bank and contained an

to be temporarily stopped. He showed a urticaria on the 2nd day, and again had a chill on the 3rd and last day of transfusion. During this period of transfusion, the temperature ranged from normal to 101° F. He complained frequently of headache and some soreness in his mouth. He also complained of feeling nauseated and vomited 3 to 4 times.

Following the period of transfusion, his temperature was normal for the 9 days he was observed.

CASE 2. C. S., 67 year old white man, admitted and operated on, April 26, 1944, because of perforated gastric ulcer (perforation occurred 4 to 5 hours previous to operation). On May 7, the patient had a left saphenous ligation.

On May 31, the patient was started on a diet consisting of hydrolyzed protein

79.2 gm., dextro-maltose 435 gm., orange juice 300 cc., ascorbic acid 200 mg., thiamin 4.5 mg., riboflavin 2 mg., niacin 40 mg., percomorphum 5 ggts., and ferric ammonium citrate 15 cc. of a 25% solution made up in 750 cc. of water. This diet provided 62 gm. of protein and 2107 Cal. The first collection period was started on June 5. During the 3rd period the patient received 1000 cc. of plasma daily, obtained from Sharpe & Dohme, which contained 7.31% protein. The plasma was administered in 2 doses morning and afternoon. The diet during this time contributed 1817 Cal.

The temperature of the patient showed little fluctuation previous to the experiment and was normal 15 days before the study. The temperature remained normal throughout the entire experiment, except on the last day of the transfusion when it rose to 100° F. for a few hours, but it rapidly returned to normal and remained so.

The patient complained of some headache and towards the end of the 3rd period, of nausea and sore gums. He vomited following the very last transfusion. However, only 0.18 gm. of nitrogen were lost by this emesis. Through an error, this subject was not given the hydrolyzed protein on the 1st day following the transfusion, which explains the lower nitrogen intake in Period 4. No untoward symptoms were noted in the patient during the remainder of the experiment (27 days).

Case 3. C. M., 20 year old Negro male, admitted to hospital and operated on, July 20, 1944, because of perforated duodenal ulcer (perforation occurred about 5 hours previous to operation).

Patient was started on a diet, August 4, consisting of 90 gm. of hydrolyzed protein, dextromaltose 539 gm., orange juice 600 cc., ascorbic acid 100 mg., thiamin 5 mg., riboflavin 3 mg., niacin 20 mg., pantothenic acid 25 mg., percomorphum 5 ggts., ferric ammonium citrate 15 cc. a 25% solution. The diet was made up in 750 cc. of water. The 1st collection period was started on August 8. The diet provided 70 gm. of protein and 2682 Cal. During the 3rd period the patient received 1000 cc. of plasma for 4 days and 500 cc. on the 5th. The last 500 cc. were omitted since the patient was not feeling well because of the frequent large transfusions. The plasma was obtained from Sharpe & Dohme, which contained 7.31% protein. The plasma was administered in 2 doses, morning and afternoon. The diet during this time contributed 2344 Cal.

The patient had an elevated temperature (between 99.4 to 101.2° F.) for 60 hours after the operation and from this time until studies were begun his temperature was normal. During the period of transfusion his temperature was normal most of the time, but on several occasions rose to 100° F. He complained of headache, nausea, vomited small amounts several times and also complained of sore gums and throat. During the 1st post-transfusion period his temperature was normal most of the time, except for 2 rises to 100° F. The temperature remained normal during the remainder of the experiment (20 days) and no untoward symptoms were noted.

Discussion. *Nitrogen Balance Studies.* Although all subjects were in positive nitrogen balance during the period of transfusion, the urinary nitrogen excretion in the post-transfusion period was increased above the 2 control periods, resulting in a loss of nitrogen. The total urinary nitrogen excretion in the post-transfusion period was very similar for the 3 subjects, but the resulting nitrogen loss was variable. This difference may have been influenced by the age of this group which varied from 20 to 67 years. Subject C. M. showed a fairly marked positive nitrogen balance previous to the transfusion which might be accounted for by the fact that the study in this individual was started 2 weeks following the operation for perforated ulcer. It seems probable that the patient was still in the recovery or anabolic phase, and this could have influenced his response to the plasma transfusion. The difference in the nitrogen intake might also account for the variation in the loss of nitrogen in the 3 subjects following the transfusion. There is some evidence that the nitrogen balance during or following transfusion may be influenced by reaction to the injected plasma, and amount of plasma transfused. The results of the present study will, therefore, be discussed under these topics.

Positive nitrogen balance during periods of plasma transfusion have been reported by various workers,[3,7,9,19,21,22,25,26,32] and in these cases no evidence of a reaction was noted. However, a negative balance in 1 experiment during the intravenous administration of plasma was reported[7] in an animal that manifested a reaction to the transfusion. An increased nitrogen excretion in the post-transfusion period was noted[26] in 1 dog which reacted unfavorably to horse serum and in another animal which showed symptoms of irritation from the intraperitoneal injections of plasma. In the present study, Subject J. M., as shown in the case history, had chills, fever and some urticaria during the period of transfusion. He showed a marked increase in creatine excretion which was also noted by Daft, Robscheit-Robbins and Whipple[7] in an animal that exhibited a reaction. Subject J. M. excreted on an average 1 gm. of nitrogen per day more than the other 2 subjects during the transfusion period. Excretion during this period may have been less and hence nitrogen retention greater had there been no reaction. Subjects C. S. and C. M. showed only minor and infrequent temperature elevations. Creatine excretion in Subject C. M. was increased in the post-transfusion period; no change in this constituent was observed in Subject C. S. Although no studies were made, it was observed that the blood pigments in the plasma were increased during and immediately following the period of transfusion, especially in these 2 subjects. Urinary nitrogen excretion during the period of transfusion was slightly higher in these 2 patients than during the time they were on the nitrogen-free diet. Since other studies[7,19,21,22,32] in which the subjects were fasted or on a nitrogen-free diet showed little or no increased excretion during the period of transfusion, the nitrogen excretion observed in C. S. and C. M. may have reached a lower level had there been no undesirable effects from the transfused plasma. All 3 patients complained of nausea, headache, and a sore mouth during the period of transfusion which subsided shortly thereafter.

It has been suggested[7] that a sufficient amount of fat and carbohydrate may be necessary for the utilization of large amounts of plasma given by vein. An increased nitrogen excretion during transfusion and a few days following was reported[7] in an animal that received 50 gm. of extrose as the sole source of calories. When this was replaced by 200 gm. of the Cowgill diet there was clinical improvement and the nitrogen excretion decreased. Although the subjects in the present experiment received no fat, caloric intake was calculated to be adequate (1800 to 2300 Cal. during transfusion period).

The first subject, J. M., received plasma from the hospital blood bank, whereas reconstituted lyophilized plasma* was used for the last 2 subjects. Dounce and Howland[8] found that when crystalline beef liver catalase had been dried by the lyophilized process that it was not crystallizable, possessed about one-third the activity of the undried material, and that its hematin iron could be reduced, indicating some change in molecular structure. It has also been reported[31] that there is a loss in clot strength of dehydrated plasma. Seegers[27] noted that the activity of thrombin was reduced after lyophilization. Although there is no direct evidence, it seems possible that the process of lyophilizing may alter the nature of the plasma proteins, and thus may have affected the nitrogen balance.

Elman and Davey,[9] from their work in dogs, suggest that the quantity of plasma injected may have an influence on the utilization of the plasma protein. In their study, most of the gain in nitrogen during the week of injection was lost in the post-transfusion period. Their injections (600 cc. per day, or an amount equivalent to 7 gm. of nitrogen) were larger than those used by other workers. In a later study these same authors,[10] from

* Kindly furnished by Sharpe & Dohme Co., Philadelphia, Pa.

work on intravenous administration of amino acids, concluded that there seems to be a ceiling utilization of nitrogen at least when given in this form. It seems plausible that there may also be a ceiling utilization of injected plasma protein. In the plasma transfusion studies on humans[21] 500 cc. of plasma were injected daily for 5 days. Muether and Andrews[25] do not state the amount of plasma injected, but their figures of 25 to 45 gm. of plasma protein would indicate a transfusion not much greater than 500 to 750 cc. These authors did not observe an increased nitrogen excretion in the post-transfusion period. In the present study, approximately twice this amount (*i. e.*, 1000 cc.) of plasma was injected daily for 5 days. Albright[2] recently reported a nitrogen balance study on a patient who was transfused with 32.1 gm. of nitrogen in the form of serum over a period of 2 days. He concluded that "15.3 gm. were metabolized as indicated by increased nitrogen in the urine and 20.1 converted into protoplasm as indicated by the decreased phosphorus in the urine. . . . The peak of the nitrogen excretion curve came on the day following the 2 days of injection and the increase of nitrogen excretion was completed by the 6th day following the injection." While the total amount of plasma transfused was less than that given to our patients, the amount given per day was larger. The increased nitrogen excretion noted by Albright following the transfusion is in keeping with the findings of the present study. Thus, it seems quite probable that the increased nitrogen excretion in the post-transfusion period was affected by the quantity transfused.

Blood Studies. The alterations in blood and plasma will be considered under plasma volume changes, protein concentration and total circulating protein, and the hematocrit and circulating red cell volume changes.

Reports on changes in plasma volume during and immediately following a transfusion are controversial. Hayward,[15] Hayward and Jordan,[16] and Sharpey-Schafer and Wallace[28] have reported a temporary increase in plasma volume in humans which was determined by following hematocrit and hemoglobin changes. The assumption that hematocrit and hemoglobin changes reflect changes in plasma volume has been criticized by Beatti.[4,5] He points out that a transfusion opens new capillaries and that some plasma can remain immobilized as a surface film without being concerned in the ratio of cells to plasma. Elman and Davey[9] using the Evans blue dye method found an increase in plasma volume in dogs at the end of a 7 day transfusion period. At the termination of the experimental period (14 to 21 days) the plasma volumes were near the control or below. Shearburn[29] noted an immediate but transient increase in total plasma volume returning to the pretransfusion level within 3 days. An increase in plasma volume following transfusion in dogs has also been noted by Freeman and Wallace[13] but no greater than was found after injection of glucose or saline. Daft, Robscheit-Robbins and Whipple[7] noted a decrease in the plasma volume during the period of plasma transfusion, but apparently felt that this was due to a deficient or inadequate protein intake. The subjects in the present study all showed an increase in plasma volume which at the termination of the experiment (9 to 24 days) approached the control value.

There was an interesting inverse relationship between plasma volume and urine excretion. The fluid intake for each individual subject was essentially the same during the control and transfusion periods, except for the additional fluid given in the form of plasma. Subject J. M., who showed an increase of 38% in plasma volume on the 1st day following the last injection, excreted during the period of transfusion an average daily urine volume of only 9% above the control values. Subject C. S., with a 16% increase in plasma volume, excreted a volume of urine 45% above the control value, and the

last subject who showed no significant alteration in plasma volume excreted a volume of urine 132% above the amount excreted previous to transfusion. Metcalf[24] in a plasma transfusion study in dogs observed that 4 to 8 hours following an injection, the rate of urine excretion correlated relatively well with the change in plasma volume.

The first subject, J. M., showed little change in protein concentration, but it was significantly increased in the last 2 subjects. The percentage increase in total circulating protein in all 3 subjects was of similar magnitude. However, as shown in Table 4, this rise was small compared to the amount injected. This observation was also noted by Elman.[10] The protein or its metabolites cannot be accounted for in the urine since urinary nitrogen was decreased at the time that the plasma protein was disappearing from the blood stream. Metcalf[24] in an attempt to determine if the protein was taken up by the liver, transfused serum directly into the portal vein. However, in this experiment, the protein appeared in the general circulation and then disappeared from the blood stream at about the same rate as in other experiments. No demonstrable change in protein content was noted in liver, muscle, kidney and intestine biopsies before and 8 to 10 hours after a large serum transfusion. The studies of Madden and Whipple[23] have indicated a rapid exchange between tissue and plasma protein which has recently been confirmed by studies[12] with the use of labelled plasma protein. But further studies are needed to give information on the storage, excretion, utilization or fate of injected plasma protein.

The change in hematocrit in the first subject was largely due to dilution since it was not accompanied by a decrease in circulating red cell volume. But the hematocrit and circulating red cell volume change in the last 2 subjects was marked. Although the method, which was used to determine the circulating red cell volume is recognized to be inaccurate,[14] the changes noted in these subjects seem marked enough to be of significance. Metcalf[24] noted a decrease in size of red cells a few hours following a transfusion in dogs. If this change occurred in these 2 patients, it could only partially account for the marked drop noted in those 2 subjects. Hematologic studies in these 2 patients showed an increase in reticulocyte count. The first patient, J. M., who received plasma from the hospital blood bank did not show the anemia. Since the last 2 subjects received lyophilized plasma, an inherent property of the lyophilized plasma was considered as a possible cause of the anemia. This plasma was preserved with phenyl mercuric borate (1:25,000), and although the amount present is so small as to be non-toxic, its possible effect was investigated by injecting comparable amounts for 5 consecutive days in 2 dogs. Blood studies on these 2 animals were followed for a period of 10 days following the last injection, but at no time was there a demonstrable change in the hematocrit or circulating red cell volume. The possibility that the process of lyophilization may alter the nature of the plasma protein has been previously discussed.

Shearburn,[29] in a study on dogs, comments that the total blood volume decreased due to an increasing anemia in 2 groups of animals given a transfusion of 50 cc. of plasma once or twice daily for 2 weeks.

While it is felt that some inherent quality in the lyophilized plasma (possibly a denatured protein) may have caused the anemia, other factors such as repeated large transfusions, cannot be definitely excluded.

Summary. Nitrogen balance, plasma volume, protein and hematocrit determinations were carried out on 3 male subjects given an adequate carbohydrate, mineral and vitamin intake by mouth and protein by plasma transfusion for a period of 5 days. Previous to, and following the transfusion, protein was given

by mouth in the form of a hydrolysate of casein. The results were as follows:

1. During the period of the transfusion, the urinary nitrogen excretion was decreased in all subjects resulting in a marked positive nitrogen balance.

2. Following this period, the urinary nitrogen excretion was increased over the control periods in all 3 subjects resulting in a loss of 13, 87 and 50% respectively of the nitrogen gained during the period of transfusion. The variation in loss among the 3 subjects may have been due to difference in age, the use of reconstituted lyophilized plasma in the latter 2 subjects, or level of protein intake following the transfusion. Reaction to the plasma transfusion did not seem to have a great effect on the nitrogen balance. There is some indication that the increased nitrogen excretion during the post-transfusion period may have been due to the quantity of plasma transfused.

3. Nitrogen excretion and partition in 2 of the subjects on a protein-free diet showed some similarity to that noted during the period of transfusion, except for the amount of creatine excreted.

4. The increase in plasma volume was variable in the 3 subjects, but appeared to be inversely related to the volume of urine excreted.

5. The increase in total circulating protein varied from 33 to 52% but was small compared to the amount of protein injected.

6. Two of the subjects showed an anemia following the plasma transfusions as evidenced by a decrease in calculated circulating red cell volume.

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PARTIAL MATURATION OF LEUKEMIC MYELOBLASTS FOLLOWING FRESH PLASMA TRANSFUSIONS

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In a case of acute myelogenous leukemia, Sabin, Austrian, Cunningham and Doan[7] found that following whole blood transfusions some of the myeloblasts in the peripheral blood were replaced by early stages of myelocytes. Following each transfusion the percentage of myeloblasts in the differential count declined, while the percentage of early myelocytes rose. After a few days, a relapse occurred and the differential count returned to its original condition, so that these cell types bore an inverse relationship during the period of investigation. It was not known if the factor causing the partial maturation of the myeloblasts was in the transfused cells or in the plasma, nor could the manner of its action be determined. Recently 2 cases of acute myeloblastic leukemia afforded an opportunity to make a further step in investigation. Both patients were in good clinical condition, in spite of the gravity of their disease, and had no need of whole blood transfusions. They were given fresh plasma transfusions to see if the partial maturation described above could be confirmed. The blood plasma was obtained by allowing the cells to settle out of the citrated blood obtained from donors, and was therefore about 2 days old at the time it was given to the patients.

The differential counts were made from dried smears stained with Wright's stain and from supravital preparations. Neutrophil granules when they first appear in the youngest promyelocytes do not stain well with the former method, or for that matter, with any of the Romanowsky stains. Supravital staining with neutral red and pinacyanole when properly done and when studied with the best available optical system under optimum conditions for microscopy makes possible considerably more accurate differential counts of the earliest cells containing specific granules. This method was therefore used in constructing the curves subsequently referred to in this paper. There is at present, however, some misapprehension about the appearance of myeloblasts and neutrophilic myelocytes A when studied in supravital preparations, because in the early work with the method[2,7] no distinction was made between neutral red vacuoles and neutrophil granules, which also stain with neutral red. In the above quoted papers, a myeloblast was defined as a cell with a basophilic cytoplasm, having a large number of mitochondria in the cytoplasm and containing no bodies staining with neutral red. Myelocyte A was defined as containing a small number of granules, not more than 10, staining with neutral red, clumped together in the cytoplasm near the centrosphere. These "granules" were thought to be the precursors of the neutrophilic granules, because they stained much more intensely than do the granules of adult neutrophils. The present writer feels that this is a misinterpretation, and that these "granules" were actually neutral red vacuoles. It will be noted in the plates of the first paper cited that the neutral red bodies are drawn as large or larger than the

* This work was done while the author was a member of the Department of Anatomy, Albany Medical College, Union University, Albany, N. Y. He wishes to acknowledge his indebtedness to Dr. Otto A. Faust, Professor of Pediatrics in the Albany Medical College, and to the Resident and Interne Staffs of his Department for the courtesy and coöperation which made an exhaustive study of these cases possible.

mitochondria. This is within the size range attained by vacuoles, whereas neutrophilic granules are very much smaller, and are just visible as separate granules when the greatest possible resolving power of the microscope is employed by immersing the condenser. The cells illustrated are therefore not myelocytes A but myeloblasts, for a cell cannot be designated as one of the myelocytes until it has acquired definite specific granules. Hall[3] was apparently the first to notice that myeloblasts may contain neutral red vacuoles. All of the myeloblasts of Case 1 of this paper contained neutral red vacuoles, and some of the cells had a large number of them: 70 to 80 per cell, in addition to the usual number of large mitochondria. The myeloblasts of Case 2, however, were apparently somewhat younger and a small number of them never acquired any neutral red vacuoles, even after several hours on the supravital slide. These vacuoles as was noticed by Cunningham, Sabin and Doan[2] were of a more reddish color than the neutrophil granules of mature cells. However, there is also a slight difference in color between vacuoles and neutrophil granules when the latter first appear around the "centrosphere" (Golgi apparatus), inside of the clump of vacuoles, as described by Simpson and Deming.[9] This slight difference in color was not noticed by the above-mentioned 3 investigators or by Hall, probably because the optical systems they employed did not have sufficient color correction and resolving power to make the difference visible. This question will be discussed more fully in another publication.

Meanwhile a myeloblast in a supravital preparation will be redefined as an undifferentiated cell normally found in the bone marrow, with a large nucleus having a fine chromatin reticulum and a colorless or faintly yellow cytoplasm which contains large scattered mitochondria and may or may not contain neutral red vacuoles, but in which there are no neutrophilic or other specific granules. Myelocyte A will be redefined as the first cell of the granulocytic line which contains a small clump of specific (neutrophilic) granules clumped around the "cytocentrum" (Golgi apparatus), the diameter of the whole clump of neutrophilic granules not being greater than 3 times the diameter of the cytocentrum. Most of these cells have an irregular ring of vacuoles around the periphery of the clump of granules, as has already been mentioned, and in addition there may be a variable number of vacuoles scattered in the cytoplasm. Myelocyte B is any cell with more than the above number of specific granules, but less than the full complement, while myelocyte C is a cell with the full complement of granules but with a round or oval nucleus to distinguish it from the later stages of the granulocytic lines. Myelocytes B and C have neutral red vacuoles as well as specific granules in their cytoplasm, but their number decreases as the cell matures.

Case Reports. CASE 1. This patient was a 6 weeks old male infant who had been born with what subsequently proved to be a leukemic tumor of the lower lip. No blood studies were done until the child was referred to the Albany Hospital for diagnosis of the tumor, at which time the leukemia was discovered. The patient was studied for 10 days. The total white blood count during the period of observation varied from 16,000 to 26,000, while the red cell count remained in the vicinity of 3,000,000 cells per c.mm. Blood platelets were reduced, but there was no purpura or other bleeding. The range of cells in differential counts made from smears stained with Wright's stain for the 1st week of hospitalization was as follows: segmented neutrophils 0 to 0.5%, band forms 1 to 2.5%, metamyelocytes 0 to 1.5%, myelocytes 0 to 0.5%, promyelocytes 0 to 2%, leukoblasts and myeloblasts 56 to 61%, monocytes 0 to 0.5%, lymphocytes 36.5 to 41.5%. Congenital leukemia is extremely rare, and this case will therefore be reported in detail elsewhere.

The results of differential counts of myeloblasts and early myelocytes made with the supravital method in this case are shown in Figure 1. It can be seen that for the 1st week, the number of myeloblasts and pro-

myelocytes (myelocytes A and B) was approximately equal, though the percentage varied somewhat from day to day. The uppermost curve on the chart shows the combined percentages of myelocytes A and B, while the lowermost one shows the percentage of myelocytes B, which for this period of time varied from 0.5 to 2.5%. The great majority of these cells had only slightly more neutrophil granules than myelocytes A. The combined curve was drawn because it is a matter of opinion whether a particular cell is a late myelocyte A or an early myelocyte B. The presence or absence of neutrophilic granules is, however, a more objective criterion and a sharp distinction between myelocyte A and myeloblasts can be made by any competent observer who has an adequate optical system and who uses it with some regard for the principles of good microscopy.

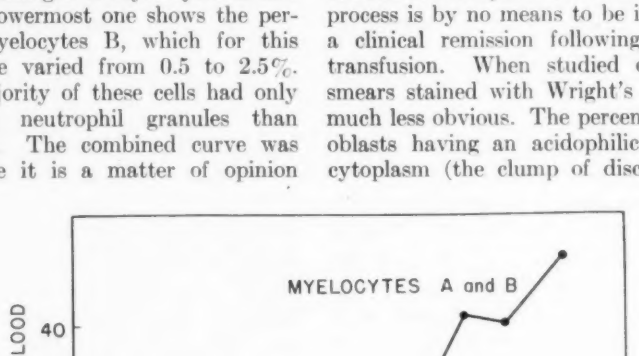

FIG. 1.—Maturation of myeloblasts produced by a fresh plasma transfusion in Case 1.

On the 7th day, a 90 cc. transfusion of fresh plasma was given without reaction. On the following 3 days the percentage of myeloblasts significantly decreased (Fig. 1), while the percentage of myelocytes A and B significantly increased. During this period the absolute number of myeloblasts decreased from 5280 per cc. to 3890, while the number of myelocytes A and B increased from 4460 to 10,340 per cc. There can be no doubt therefore that the fresh plasma transfusion produced a partial maturation of some of the myeloblasts. This maturation process is by no means to be interpreted as a clinical remission following the plasma transfusion. When studied on the dried smears stained with Wright's stain, it was much less obvious. The percentage of myeloblasts having an acidophilic spot in the cytoplasm (the clump of discretely visible neutrophil granules in the supravital preparations) increased somewhat, and those with a uniformly basophilic cytoplasm were somewhat fewer after the transfusion. It is, however, very difficult to decide whether some cells have or do not have a very faintly acidophilic spot, and impossible to place much confidence in observations in which the subjective element is so great. The number of myelocytes A always exceeded the number of myeloblasts having an acidophilic spot in the cytoplasm, showing that neutrophilic granules do not stain in Romanowsky preparations for some time after they can be demonstrated with neutral red in the living cell.

CASE 2. This patient was a well-developed, well-nourished 13 year old white school boy who was admitted to the hospital with complaints of fever, cough, with blood-streaked sputum, swelling of the neck and malaise of 3 days duration. There were areas of petechiæ in the axillæ and groins and scattered petechiæ on the abdomen and chest. Bluish subcutaneous nodules about 0.5 cm. in diameter were scattered over the cheeks, neck, arms and thighs. The gums were hypertrophied, tender, ulcerated and bled easily. The pharynx was injected, the tonsils large and the left one covered with a necrotic yellowish membrane. There was generalized lymphadenopathy and the spleen was palpable 3 cm. below the costal margin. The liver was not palpable. The blood count on admission showed 13 gm. (88%) hemoglobin, 4,350,000 red blood cells and 34,400 white blood cells per c.mm. The differential count on admission was segmented neutrophils 6%, lymphocytes 5% and large, primitive looking cells having nucleoli 89%. The latter cells were subsequently identified as myeloblasts. The patient was studied for 3 weeks. During the period of hospitalization the hemoglobin varied from 10 to 13.5 gm., and the red blood count from 3,480,000 to 4,600,000. The white cell count after admission rose to 75,900 in the 1st week, declined to 28,100 at the end of the 2nd week, and then rose again to 48,000 on the day of final discharge. The range of the neutrophilic cells in the differential counts was as follows: segmented neutrophils 1 to 6%, band forms 0.5 to 3.5%, metamyelocytes 0 to 2%, myelocytes 0 to 0.5%, promyelocytes 0 to 2.5%, and (with Wright's stain) myeloblasts 77.5 to 89%. The lymphocytes varied from 5 to 16.5%, and occasional eosinophils and basophils were found. No monocytes were seen in this case either with Wright's stain or with the supravital method.

In Case 1, the myeloblasts when stained with Wright's stain all contained considerable numbers of azure granules, and when studied in the supravital preparations, the number of myeloblasts and myelocytes A were approximately equal as has been described. In this case, however, the cells were much more primitive, and when stained with Wright's stain during the 1st week the patient was under observation, only 8.5 to 15.5% of myeloblasts contained azure granules, and at no time did more than 26.5% of them show azure granulation. When studied with the supravital method during this time 70 to 80.6% of the cells in the differential count were myeloblasts and only 5 to 12.5% were myelocytes A (Fig. 2). No myelocytes B were seen during this period, and only a small number of them subsequently. They have therefore been left off of the chart. Peroxidase positive cells made up 5 to 8.5% of the differential count.

On the 8th hospital day, the patient was given a 400 cc. transfusion of fresh plasma, without reaction. Following this the number of myelocytes A rose to 20.5%, but within 3 days after the transfusion declined again to 12%. A second fresh plasma transfusion was then given, followed by a rise of myelocytes A to 26% in 24 hours, and then a decline to 10.5% in the 3 days following the transfusion (Fig. 2).

The patient was then taken home by his family, but returned to the hospital 3 days later, somewhat dehydrated, complaining of pain from his cough and difficulty in swallowing which made feeding him at home impossible. After these complaints had been relieved as much as possible by suitable medication, the patient was given 2 intramuscular injections of 17% gamma globulin solution to see if the substance which produced the partial maturation of the myeloblasts is present in that plasma fraction. The first injection was made with 10 cc. into either buttock, the second with 15 cc. into either buttock 24 hours later. Each intramuscular injection was preceded by 2 cc. of 0.5% novocain through the same needle, and produced a minimum amount of discomfort to the patient. This amount of gamma globulin was given upon the assumption that 25 cc. of this solution would contain about the same amount of gamma globulin as a 500 cc. fresh plasma transfusion. These injections were, however, without effect on the differential count (Fig. 3). The following day the patient was given a 250 cc. transfusion of commercial dried plasma ("Lyovac"), made up so as to contain as much protein as 500 cc. of fresh plasma. This also had no effect on the differential count (Fig. 3).

On the 5th day after his second admission the patient was again taken home by his

family, and died a few days later. No autopsy was obtained.

COMMENT. Leukemic cells have been grown in tissue culture with a variety of media and methods.[1,5,6] All authors who have studied the problem agree that there are no marked differences in behavior between leukemic cells and normal cells, and that the younger cells in the cultures mature to older forms. It is reported that myeloblasts will develop granules and eventually transform into adult granulocytes. In acute myeloblastic leukemia, relatively few of them seem to be able to accomplish

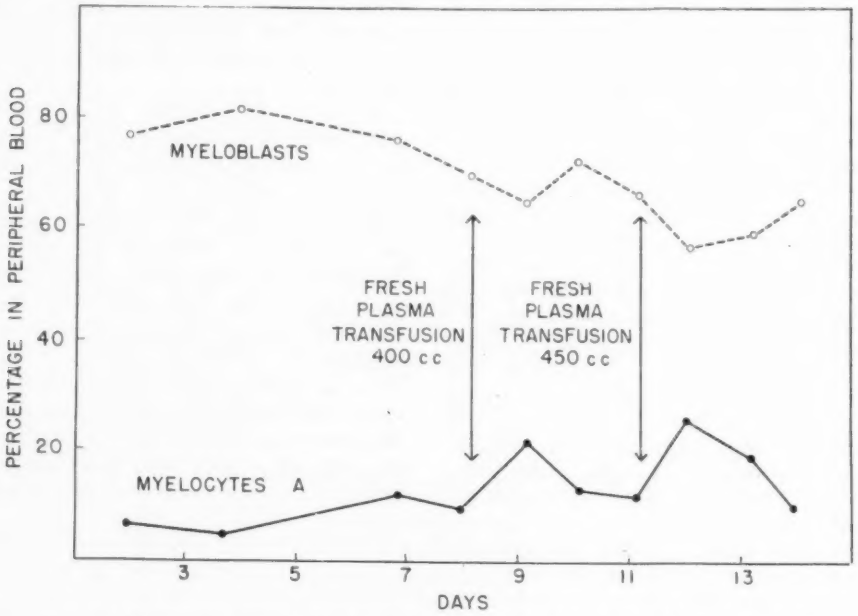

FIG. 2.—Maturation of myeloblasts produced by fresh plasma transfusions in Case 2.

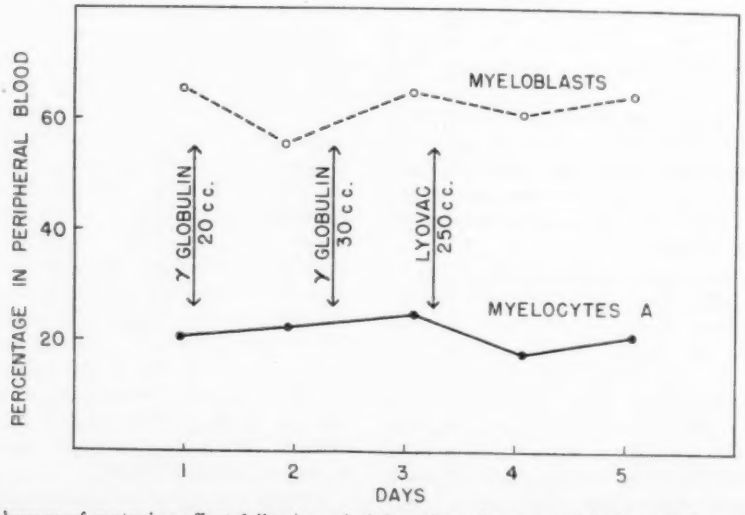

FIG. 3.—Absence of maturing effect following administration of gamma globulin and Lyovac plasma.

this change in the body of the patient. It is possible that an inhibitor substance is present, which prevents the maturation of the myeloblasts. It would seem more likely, however, that there is some substance missing from the environment of such cells while they are in the body, the lack of which prevents their maturation. Evidence of this was obtained by Timofejewsky and Benewolenskaja,[10] and more recently by Houghton[4] in experiments in which myeloblasts of acute myeloblastic leukemia were grown in tissue cultures with normal plasma as the medium. After a time, the cultured myeloblasts began to mature. This significant finding is confirmed by the present experiments, which show that fresh normal blood plasma will produce a maturing effect on the myeloblasts of acute myeloblastic leukemia *in vivo*, just as it does in tissue culture. The maturation produced in the patient is, however, only partial and is moreover of a transitory nature, possibly because the substance producing it is present in very small amounts in the plasma and is quickly used up. There is a limit to the amount of plasma which can be given to a patient without getting into serious difficulty with the fluid balance. In tissue cultures, the appearance of specific granules in the cultured myeloblasts takes place in a matter of hours. It is realized that much more frequent differential counting of these cases would have been desirable, but the exigencies of a war-time teaching schedule made this impossible. A preliminary note on this work has been published.[8]

The essential results of Sabin, Austrian, Cunningham and Doan, namely that whole blood transfusions cause maturation of the myeloblasts of acute myelogenous leukemia, are confirmed by the present work, even though, as has been shown, it is impossible to be sure which cells they were counting as the more mature forms. It has been further shown that the maturing substance is carried in the plasma, and is not some factor liberated by the breakdown of transfused blood cells.

The substance which causes the maturation of the myeloblasts is not present in the gamma globulin fraction of the plasma, at least in the form in which this fraction is currently available. It is not present in ordinary dried plasma, if 1 test is a reliable criterion. Possibly it is destroyed during the processing and subsequent dehydration. It need hardly be added that the nature and amount of this substance present in fresh plasma are completely unknown.

Summary. 1. Fresh plasma transfusions resulted in the partial maturation of myeloblasts in 2 cases of acute myeloblastic leukemia.

2. The results of other authors with whole blood transfusions are thus confirmed.

3. Fresh plasma will cause the partial maturation of leukemic myeloblasts *in vivo* as well as *in vitro*.

4. The substance causing the maturation is not present in the gamma globulin fraction of blood plasma or in dried plasma.

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GYNECOMASTIA ASSOCIATED WITH VITAMIN DEFICIENCY DISEASE

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An outbreak of gynecomastia was observed among the Americans held as Japanese prisoners-of-war in the Philippine Islands. This group of 7000 to 8000 surrendered on Bataan April 9, 1942, and was liberated Jan. 30, 1945. During these 34 months malaria, dysentery and vitamin deficiency disease attacked almost every man in camp.[2] The prisoners were always underfed, suffered from all the avitaminotic states, acute and chronic bacillary and amebic dysentery, and malaria.

The appearance of enlarged breasts did not closely coincide with the onset of any deficiency disease Nutritional edema, pellagra, ariboflavinosis, beriberi, peripheral neuritis, scurvy, xerophthalmia and corneal ulceration had their onset in approximately that order between April and December 1942. The first cases of gynecomastia were seen in November 1943, or after 1½ years of prison life, almost 1 year since the appearance of a new deficiency disease. During this period there was a relative increase in diet providing approximately 1200 to 1400 Calories and a corresponding decrease in the incidence of malaria and dysentery.

The census of the camp at this time was about 5000, of which 500 (10%) had breast disturbances. They were of military age, almost all between 20 and 30 years. This group did not necessarily represent the severest cases of malnutrition, although there was usually a history of many deficiency diseases. Almost all of the men had gained from 10 to 50 pounds in the few months just prior to the "epidemic" of gynecomastia. There was no particular type of habitus among the involved men.

The onset of gynecomastia was insidious but simultaneous in this group. It is impossible to say how long the process had been active, but within 2 to 3 weeks a small tumor appeared. Both breasts were involved, usually to the same degree, and the size was comparable to the breast of an adolescent girl. An irregular, firm, nodular mass, attached to the nipple but freely movable over the deep structures, could be felt. This was usually not tender except after trauma. Thin, slightly milky fluid was expressed in a few cases, but this was not the rule. The maximum size of 2.5 to 3.5 cm. in diameter was reached in 2 to 3 months, and regression was complete in about another 3 months.

A few men continued to have nodular breasts, although small, even until liberation 1 year later. There were no changes in hair distribution or pitch of voice. The size of testicles almost always remained unchanged, although 5 or 6 men had lost a testicle during the period of vitamin A deficiency, due to suppurative orchitis. Symmetrical enlargement of one or both testes was observed in rare cases. Potency and sexual desire and nocturnal emissions were not noticeably changed, having been at a very low level before gynecomastia appeared. Sperm counts were not obtainable. The rest of the examination revealed severe malnutrition, residual signs of neuritis and pellagra.

Weekly examinations were done on a group of 100 men with gynecomastia. The course was one of steady progression and then regression, completed within 6 months after onset. We became satisfied that the lesion was self-limited, and no treatment was suggested. Improvement coincided with a shipment of Red Cross food and sufficient vitamins to pro-

vide each man with 1 vitamin capsule* daily beginning in January 1944. A small number of men continued to show a progressive enlargement of the breast after 6 months. These breasts were surgically removed. Macroscopically there appeared to be small projections of tissue in the ducts, intracanalicular fibro-adenomas. No facilities were available for microscopic examination.

Discussion. A causal relationship was never definitely established, but the most obvious explanation was a disturbance due to vitamin deficiency. We thought that the severe prolonged malnutrition had temporarily upset the endocrine balance. There was no clinical evidence of degeneration of the liver which might have resulted in an excess of estrogen in the systemic circulation.[1]

It is interesting to note that shortly after the disappearance of the gynecomastia we discovered blood pressure readings above 140 mm. systolic and 90 mm. diastolic in about 60% of the entire personnel. No attempt was made to check the blood pressure of those patients who had recovered from gynecomastia. The hypertension was transitory, lasting from 4 to 6 months, and adrenal dysfunction was the suspected cause.

Conclusions. 1. About 500 cases of gynecomastia were seen in males after severe, prolonged malnutrition. It appeared many months after the onset of any of several deficiency diseases, while there was a relative increase in diet.

2. There appears to be a relationship between endocrine balance and adequate vitamin intake.

3. Transitory hypertension may be further evidence of this relationship.

* These capsules were vita-kaps, each containing Vitamin A, 5000 U.S.P. units; Vitamin D, 500 U.S.P. units; Vitamin B_1 (thiamine), 333 U.S.P. units (1 mg.); Vitamin C, 600 U.S.P. units (30 mg.); Vitamin G (riboflavin) 2 mg.

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THE EFFECT OF PATENT DUCTUS ARTERIOSUS ON BODY GROWTH*

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Review of key publications[1,3,4,5,6] dealing with various aspects of persistent patency of ductus arteriosus reveals no essential concern with the effect of this congenital vascular lesion on body growth.

Evaluation of the effect of any one factor on body growth is difficult because of the complexity of growth influences in general, but the problem is to a degree simplified by the nature of the physiologic disturbances concerned in patency of a ductus arteriosus. Most if not all elements influencing growth are transported to the tissues of the body through the blood stream. It is reasonable to infer that a lessening of the quantity of blood reaching the tissues, or a reduced concentration in the blood of growth promoting elements, will influence the rate of body growth. And if one or both influences persist through the growth period, stunting will result.

The degree of patency and the consequent dynamic alteration of the circulation varies greatly in magnitude. The shunt of blood from the aorta to the pulmonary artery may vary from a few cc. to 70%[2] or more of the output of the left ventricle. Manifestly in many patients the normal quantity of blood delivered to the left ventricle by the right ventricle is shunted from the aorta so that physiologically sufficient quantities do not perfuse the body tissues; the deficit depending upon the size of the patency and the capacity of compensating mechanisms.

One is rarely impressed with the smallness (height and weight) of many patients who have patent ductus arteriosus until he observes them in very striking contrast of body size with their parents, brothers, sisters, and children.

This study is concerned with 3 patients. The charts are designed to show the contrasting physical size, height, weight, and body surface area in square meters. No factor other than the patent ductus could be found to account for the smallness of each of the 3 patients, and when the ductus was successfully closed in one, he outstripped his twin in height in less than 3 years.

Case 1. (Fig. 1) a female, aged 23, had a ductus 1 cm. in diameter at operative ligation. This patient had gross cardiac enlargement and early congestive failure. She was considerably shorter and less heavy than her parents, brother, and sisters.

Case 2. (Fig. 2) a mother, aged 34, is considerably shorter and less heavy than her parents, brothers, sisters, and her own child, aged 15.

Case 3. (Fig. 3) one of identical male twins, aged 11, the other normal. The one with a gross patency was both shorter and lighter than his twin; 35 months after surgical cure, he had grown 16.4 cm. which was 6.1 cm. more than the growth of the normal twin.

Discussion. At the beginning it was admitted that the evaluation of growth inhibiting factors is difficult, yet a study of the charts is convincing that a factor did exist in these patients which inhibited the anticipated genetic growth influences.

All 3 of the patients enjoyed good home environments, had no peculiar food habits, and economically were in the upper strata of society.

They had each been peculiarly free from infections and from a physical standpoint were normal except for body size and the cardiovascular defect.

None of the patients were interested in sports requiring physical activity. They

* Presented at the 59th Meeting of the Association of American Physicians, May 1946.

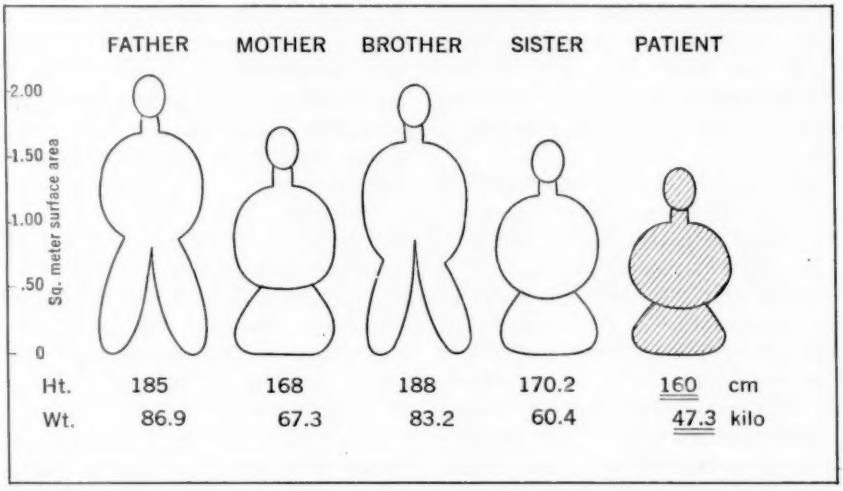

FIG. 1.

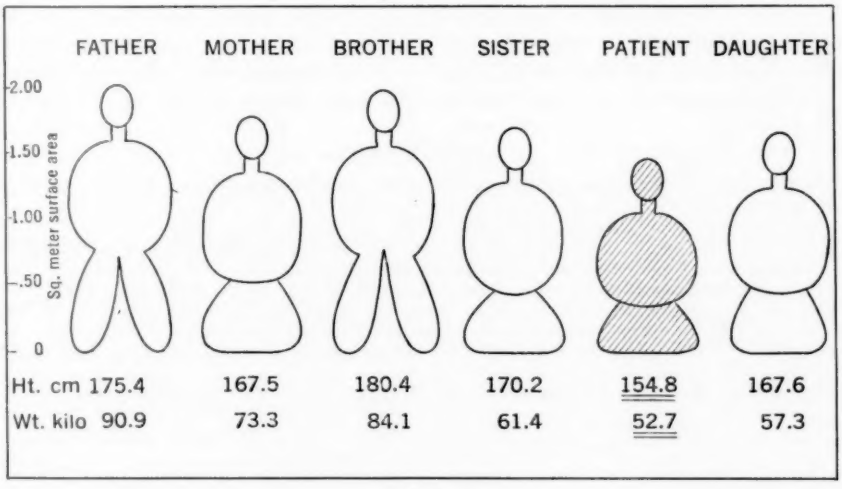

FIG. 2.

	PATIENT		BROTHER	
	Ht. cm	Wt. kilo	Ht. cm	Wt. kilo
May 1943	146.1	32.2	149.8	35.9
	Operation			
April 1946	162.5	40.9	160.1	45.5
	Growth in 3 years 16.4 cm		Growth in 3 years 10.3 cm	

FIG. 3.

stated that they simply did not enjoy such exertion; however it is more likely that they had been inhibited by physical limitations. Non-participation in sports is not a logical explanation for the stunting of growth.

In this connection it is interesting to comment on the twins. Before operation the twin with the patent ductus took little exercise. Since his recovery he has become even more active physically than his normal twin brother. This may be an explanation for the fact that his weight has not kept pace with the growth in height. Skeletal growth, after all, is much more significant in a youth of the patient's age than the accumulation of fatty tissue.

Finally it must be emphasized that if a patent ductus arteriosus retards body growth its effects will be clinically impressive only in those patients in whom the circulatory shunt is of considerable magnitude. Even in these patients compensatory adjustments may in some instance so nullify the effects that growth is normal. It is doubtful therefore, if there is an absolute ratio existing between the size of the patent ductus and the consequent growth inhibition for the complexities of biological results cannot be predicted by mathematical equations.

Summary. The charts show these patients to have been definitely influenced by some factor resulting in positive inhibition of body growth. The only apparent and consistent influence is that of patent ductus arteriosus, presumably due to the lessening of the normal supply of blood to the body tissues.

The rapid increase in height observed in the twin after operative closure of a patent ductus is impressive and convincing.

In many patients the growth inhibiting factor is an added reason for surgical ligation of a patent ductus arteriosus.

The optimum period for operation is probably before the age of 11 years.

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HEBERDEN'S NODES

VI. THE EFFECT OF NERVE INJURY UPON THE FORMATION OF DEGENERATIVE JOINT DISEASE OF THE FINGERS

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Heberden's nodes are enlargements of the terminal interphalangeal joints of the fingers due to degenerative joint disease. Although the process always starts in one finger it soon spreads to others until several fingers of both hands are affected. It is the purpose of this presentation to describe a series of cases in which Heberden's nodes failed to appear in one hand which was the site of serious trophic nerve disorder although deformities had developed to a severe degree on the healthy or non-paralyzed side.

Isolated nodes occurring in a single finger, the result of direct injury and most often seen in males, do not spread to uninjured digits and are not to be confused with the so-called idiopathic Heberden's nodes with which the present discussion is particularly concerned. Former studies indicate that the formation of idiopathic Heberden's nodes is influenced by sex, age and race, being most common in white women after the age of the menopause.[8] The most important factor so far recognized in etiology is heredity.[9,10] The hereditary influence was indicated by observing that the mothers of affected women were involved twice and the sisters 3 times as often as women in the general population. The distribution of involvement among family pedigrees together with gene frequency analysis supported the hypothesis that the genetic mechanism of idiopathic Heberden's nodes involves a single autosomal gene, sex influenced, dominant in females and recessive in males.

Although susceptibility to this condition depends upon genetic and constitutional factors, clinical evidence indicates that Heberden's nodes develop only in a hand which has an intact nerve supply, which functions normally and in which the tissues are not subject to trophic disorder. The lesion fails to develop or is retarded in its progress in the presence of either peripheral nerve damage, spinal cord disease or palsies of cerebral origin. This fact is demonstrated by 3 cases we have observed and are describing herewith and by 9 cases described in the literature and by personal communications. The first case is one of a woman with a peripheral nerve lesion.

Case Abstracts. Case 1. Mrs. E. V., a 53 year old white woman, was first observed in 1939 because of enlargements of the finger joints. There was no history of joint disease in the parents. The patient was the fourth of 8 children, 5 of whom are living. The oldest sister, age 61, reported by correspondence that her right forefinger became enlarged 4 years before and that the joints of all the fingers of the right hand and the forefinger of the left hand are now enlarged. Another sister, age 56, reported by correspondence an enlargement of several fingers of 2 years duration.

Past History. There were no serious illnesses or major operations. At the age of 41, 12 years ago, the patient sustained a deep laceration from a broken milk bottle in the left palm through the thenar eminence resulting in a paralysis of the index and middle fingers and a partial paralysis of the thumb, ring and little fingers. The menses stopped at the age of 50 years.

since which time she has had frequent hot flashes.

Present Illness. In the right or uninjured hand the forefinger began to enlarge at the age of 50, 3 years ago, since which time all the other fingers of the same hand became similarly involved. The enlargement of the joints which were originally involved continues to increase but at no time has there been any pain or serious discomfort. In contrast only the little finger of the lacerated hand developed joint enlargement, the other fingers having escaped this involvement. Disease of other joints, particularly of the large articulations, has not been demonstrated.

FIG. 1.—Mrs. E. V. Right hand shows enlargement of the terminal joints of the index and little fingers. In the left hand, the index and middle fingers are thin and tapering. Skin on these fingers is thin, glossy and free of wrinkles. The index finger shows a flexion deformity of the proximal joint while the middle finger is stiffened and straight. The median nerve of the left hand was injured by a laceration 12 years before.

Physical Examination. In the right hand where no nerve injury occurred, the terminal joints of the fingers are enlarged (Fig. 1). This is particularly prominent in the index and little fingers both of which are further deformed by deviation. The normal wrinkling of the skin is clearly marked, especially over the proximal interphalangeal joints. There is no sign of motor weakness and the patient can make a tight fist with a normal closure.

In contrast, the left hand in which there was obviously an injury to the median nerve, shows striking differences from the above. Instead of the typical bulbous appearance of Heberden's nodes, the index and middle fingers of this hand are thinner, have a tapering appearance and seem to be actually longer than their mates. The skin is thin, glossy and relatively free of wrinkles. The index finger shows a flexion deformity of the proximal finger joint, while the middle finger is stiffened and straight. The flexion deformity of the terminal joint of the ring finger can be corrected by passive motion.

A neurologic study of this injured hand indicates that the laceration resulted in a section of the distal sensory and motor branches of the median nerve. There is marked atrophy of the thenar muscles. The thumb fails to oppose or flex fully in the closure of the fist. Complete active flexion of the index and middle fingers is impossible because of paralysis of the first and second lumbricals. Anesthesia or hypoesthesia is present along the entire palmar surface of the middle finger and over the distal one-third of the index finger.

Roentgen Ray Studies. In the right or uninjured hand there is a marked lateral enlargement of the proximal end of each distal phalanx with apparent shortening of this bone (Fig. 2). The distal end of each second phalanx shows a bulbous enlargement involving the terminal third of its length most marked in the index finger. Joint surfaces are not distorted. There is a very small spur on the ulnar side of the proximal end of the fifth terminal phalanx. Other joints of the right hand are apparently normal.

Case Summary. A patient, 53 years old, who had a hereditary predisposition to Heberden's nodes, suffered a median nerve injury of the left hand 6 years before the actual appearance of such nodes. In the area ordinarily supplied by the injured median nerve the skin suffered trophic changes and Heberden's nodes failed to

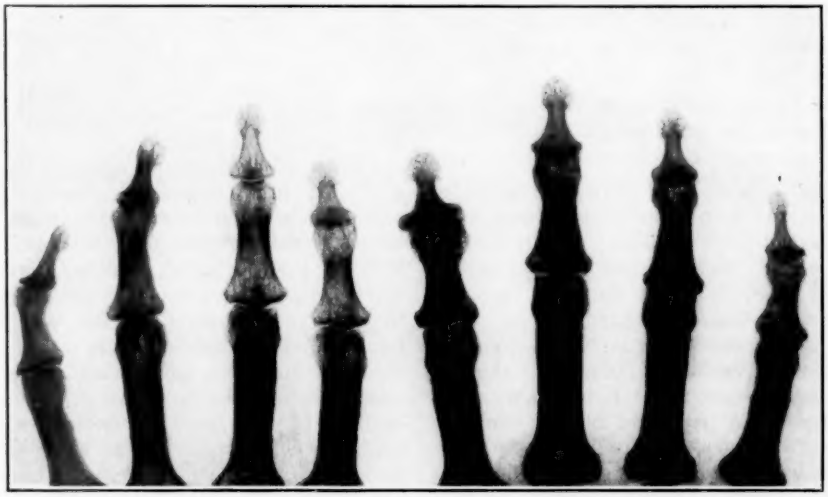

FIG. 2.—Mrs. E. V. Roentgenogram of the fingers of the right hand show marked lateral enlargement of the proximal end of each distal phalanx with apparent shortening of this bone. The distal end of the second phalanx shows a bulbous enlargement involving the terminal third of its length most marked in the index finger. Joint surfaces are not distorted. There is a very small spur on the ulnar side of the proximal end of the fifth terminal phalanx.

Roentgenogram of the left or injured hand reveal marked demineralization of the index and middle fingers being most prominent in the middle and terminal phalanges of these two fingers where the overlying tissues are supplied exclusively by the sensory branches of the median nerve. Flexion deformity, a non-compact bone structure and bulbous enlargement of the terminal joints, signs of mild Heberden's nodes, are evident in the ring and little fingers whose nerve supply is predominantly from the ulnar nerve.

Roentgen ray studies of the left or injured hand reveal a marked demineralization of the index and middle fingers. This demineralization is sharply limited, being most prominent in the middle and terminal phalanges of these two fingers, where the overlying tissues are supplied exclusively by the sensory branches of the defective median nerve. Flexion deformity, a non-compact bone structure and a bulbous enlargement of the terminal finger joints, the signs of mild Heberden's nodes, are clearly evident in the ring and little fingers whose nerve supply is predominantly from the ulnar trunk.

develop in those bones which derived sensory nerve filaments from the injured nerve alone. Nodes did, however, appear in the index and little finger of the uninjured hand and were apparent in the ring and fifth finger of the lacerated hand. Demineralization and failure of node formation was not so pronounced in phalanges which have a double nerve supply, *i. e.*, where the radial or ulnar nerves overlap the median nerve distribution. This sug-

gests that trophic bone disease depends largely upon damage to the sensory nerve supply to the joints and to the periosteum as well as to modification of blood supply.

McEwen[7] mentioned 3 patients with unilateral Heberden's nodes. In the hand where they did not occur there had been peripheral nerve injuries with obvious trophic changes in the skin. In 1 of these patients, 3 fingers were useful and 2 were paralyzed in the injured hand. There were marked Heberden's nodes on the intact hand, moderate formations on the functioning fingers of the injured hand but no sign of nodes on the paralyzed fingers.

The second case is one of a woman with a lesion of the spinal cord.

CASE 2. Mrs. L. M., a 68 year old white woman was first seen in 1939 because of pain in the right shoulder. Family history revealed no record of joint disease except in the patient's eldest daughter, age 44, who was found on examination to have well-marked enlargements of the terminal joints of all the fingers except the thumbs diagnosed as idiopathic Heberden's nodes.

The *past history* revealed infantile paralysis in early childhood which affected the right arm leaving it smaller and weaker than the left but with no definite paralysis of any one muscle group.

Present illness began 6 years before at the age of 62 with enlargement of the terminal phalanx of the left forefinger. The left little finger became involved only within the last year. Neither of these deformities could be attributed to local trauma.

Shortly after these enlargements were first noted on the left hand, at age 65, the patient sustained a trivial injury to the right elbow, which was followed shortly by pain and stiffness of the right shoulder. The shoulder was later found to be ankylosed and consequently was manipulated under anesthesia. This procedure was followed by immobilization in abduction for several days. Two months later when she was examined, the right arm and hand were painful and useless. The pain and swelling were so severe that an erroneous diagnosis of gonorrheal arthritis had been made. The hand was swollen, the skin was shiny, cyanotic, cold and mottled in appearance. The wrist was fixed in partial flexion, the fingers were extended and passive motion was greatly restricted and painful. The sensory findings consisted of an hypoesthesia in the fields of the median and ulnar nerves. The motor and sensory defects suggested a mild combined palsy of the median and ulnar nerves.

Roentgenograms taken at the time of her first visit showed marked demineralization and other features typical of Sudeck's bone atrophy in the injured hand and arm. Definite deformity of the left forefinger due to early Heberden's nodes was seen at this time. The median and ulnar nerve paralysis was vigorously treated with heat, massage, active and passive exercise, so that both right arm and hand have regained their former degree of limited function.

Photograph of the hands taken 27 months after her first visit shows the right or atrophied hand to be the same length but definitely more slender than the left. The skin of the partially paralyzed hand is thinner, tighter and shows a marked loss of wrinkling as compared to the left. The terminal interphalangeal joints of the once paralyzed hand are quite small, giving the fingers an appearance of constriction. In contrast the forefinger of the left or normally developed hand shows enlargement, flexion and ulnar deviation of the terminal joint and normal skin wrinkling. The middle finger, as well as the little finger, presents the same bulbous deformities and only the ring finger of the uninjured left hand appears to have escaped the joint deformities characteristic of Heberden's nodes.

Reëxamination 6 years later, at age 73, shows the right or partially paralyzed hand to be essentially unchanged in appearance or function. The left or arthritic hand (Fig. 3) shows increased enlargement of all the terminal joints. There are enlargements and lateral deviation of the index, middle and little fingers and enlargement of the ring finger.

Roentgen ray studies of both hands taken at the time of the first visit, 27 months later and finally in 1946, 9 years after the first visit all show marked generalized demineralization throughout the entire bony structure of the palsied hand. The joint surfaces are everywhere smooth and regular

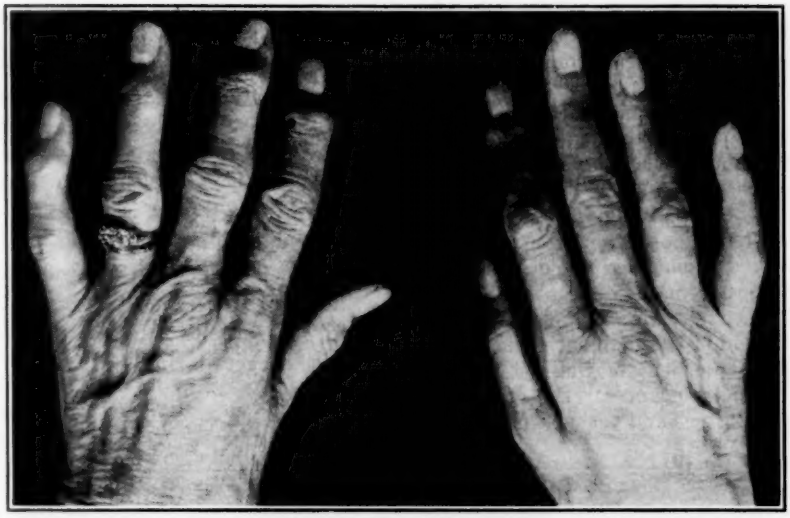

FIG. 3.—Mrs. L. M. The right hand has been seriously affected by infantile paralysis, being definitely atrophied as compared to the left. The skin is thinner, tighter and smoother than on the left hand. The terminal interphalangeal joints are small and appear to be constricted. The left or normally developed hand shows enlargement and flexion deformity of all the terminal joints. The index, middle and little fingers show lateral deviation also.

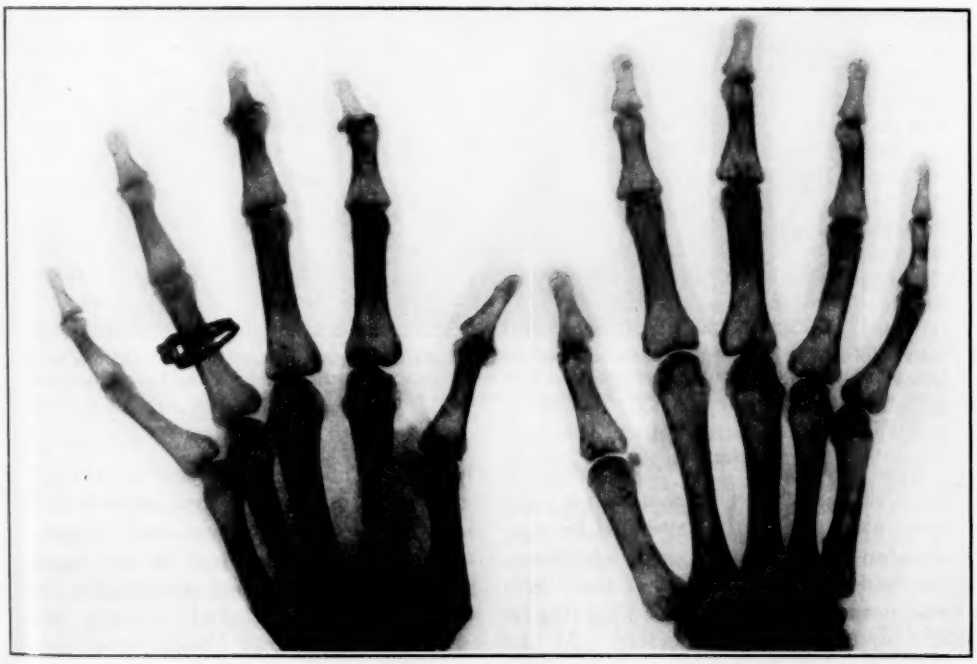

FIG. 4.—Mrs. L. M. Roentgenogram of the right or palsied hand shows marked generalized demineralization of all bones and extreme thinning of the cortex. The shape of the bones and the contour of the joints are absolutely normal. Roentgenogram of the left hand shows deformity of all the terminal interphalangeal joints. There is decrease of joint space, irregularity of joint line, broadening with spur formation of the proximal end of the distal phalanges and bulbous enlargement of the distal portion of the second phalanges. The other joints of this hand are normal.

and the joint spaces are of normal width. Little or no change is seen in this hand during the period of observation.

Roentgenograms of the left, the unparalyzed or arthritic hand at the time of the first visit, showed arthritic deformity in the terminal joints of the index and little finger. The other fingers were apparently not affected. At the time of the last visit, 6 years later (Fig. 4), the terminal joints of all four fingers show decrease or obliteration of joint space, marked irregularity of joint line, broadening with spur formation of the proximal end of the terminal phalanges and bulbous enlargement of the distal portion of the second phalanges. The other joints of this arthritic hand are normal.

and swelling with Sudek's bone atrophy of the entire right arm. Even at that time, however, definite joint deformity of the left forefinger of the other hand was apparent by Roentgen ray. The preexisting infantile paralysis with consequence trophic bone disturbance undoubtedly prevented the formation of Heberden's nodes. Nine years after her peripheral nerve injury there was still no evidence of Heberden's nodes on the right hand. Heberden's nodes on the left hand became larger during this period of observation.

Hench[5] described an elderly woman

FIG. 5.—Marked Heberden's nodes on the fingers of the right hand only. The left hand has been spared. Patient had infantile paralysis in childhood resulting in partial paralysis and lack of development of the left hand. Photograph and case history by courtesy of Philip S. Hench, M.D., and the Mayo Clinic.

Case Summary. This is a woman 68 years of age when first seen whose right hand was imperfectly developed because of infantile paralysis in early childhood. She had Heberden's nodes of the otherwise normal left hand whose joint disease first appeared 6 years before. At the time of her first visit she had a painful right shoulder which had been manipulated and after which she suffered a mild but definite combined paresis of the right median and ulnar nerves, painful stiffness

who had infantile paralysis as a child with resultant partial paralysis of 1 arm and hand. Despite persistent atrophy and arrested development of this hand, she had slowly regained considerable use of it, so that functional recovery was approximately 50%. Years later definite Heberden's nodes developed on the fingers of her normal hand but no evidence of them appeared on the partially paralyzed extremity (Fig. 5). Beard[1] reports having seen an elderly woman who

suffered infantile paralysis as a child which left her with impaired development and function of the left arm. She had marked Heberden's nodes of all fingers of the right hand when first seen 10 years before her death. There were no Heberden's nodes of the fingers of the left hand.

It has been observed that hemiplegia due to cerebral apoplexy may prevent the development of Heberden's nodes, though they become well marked on the normal side. Forestier[3] described a 55 year old woman with a right hemiplegia of 7 years duration who developed Heberden's nodes on all the fingers of the non-paralyzed hand about 8 months after her stroke. His communication included excellent photographs and Roentgenograms of both hands.

The same author presented a second woman who suffered a right hemiplegia due to hypertension at 59 years of age. For 2 years before the attack of apoplexy she observed nodosities on both thumbs and left forefinger. Since the hemiplegia, the degree of arthritis of the right thumb has remained stationary and there has been no extension to the other fingers of the paralyzed hand. However, in the left hand the arthritis has extended to the distal joints of the middle and ring fingers and the proximal joints of the second, third and fourth fingers.

Coste and Forestier[2] described a 66 year old woman who had a right-sided hemiplegia with facial paralysis and aphasia 9 years before. She had had pain and deformity of the right thumb 10 years before the stroke. One year after the stroke of paralysis, the pain, swelling and stiffness attacked all the joints of the non-paralyzed hand. The process had progressed until she presented a complete but unilateral picture of advanced Heberden's nodes. The right or paralyzed hand remained free of joint disease.

The same authors report a man with hemiplegia of 10 years duration who developed typical advanced Heberden's nodes on the sound side but little or no evidence of joint disease in the hand on the paralyzed side.

It is seen that Heberden's nodes have failed to develop in the presence of lesions of the peripheral nerves, spinal cord or upper motor neuron lesion. The question arises as to the effect such lesions might have on Heberden's nodes already present. One such case has been observed.

Case 3. Mrs. C. C. was first seen at the age of 50 with well-developed Heberden's nodes on all fingers of both hands. The enlargements increased in size considerably over a period of 2 years. She suffered a cerebral accident which left her with partial paralysis of her left side. Photographs and Roentgenograms of both hands were made at the ages of 52 and 53, 15 months after her apoplexy. Before the stroke both hands were quite similar. Photograph of the left hand at age of 52 showed marked enlargement of all the terminal interphalangeal joints with flexion deformity and deviation of the index and little finger. Photographs of the same hand after the stroke shows that the enlargement of the joints have become much less marked, the fingers have become smaller and decreased in diameter, the skin has become tight and smooth and it has lost much of its normal wrinkling. The fingers show mild flexion deformities (Fig. 6).

Roentgenograms of both hands at age of 52 show marked enlargement of the proximal ends of all distal phalanges and bulbous swelling of the distal ends of the second phalanges. There is irregular spur formation. The joint surfaces are distorted and uneven, the joint spaces are decreased in width. Because of flexion deformities they seem to have disappeared entirely in places. Roentgenogram 15 months after the stroke shows a marked demineralization throughout all bones of the left or paralyzed hand but the size, shape and outline of the bones remain absolutely unchanged (Fig. 7).

Case Summary. A 53 year old woman with well-developed Heberden's nodes in both hands was observed before and 15 months after a left-sided hemiplegia. A comparison of photographs and Roentgenograms of the hands before and after the

stroke show that the Heberden's nodes became much less marked and prominent than they had been before. This apparent recession of the process was due entirely to shrinkage of the skin and subcutaneous tissues because the size and contour of the bones remained unchanged, although marked demineralization had occurred.

Discussion. The idiopathic Heberden's nodes affecting multiple fingers of both hands occur spontaneously in susceptible people after middle life. It was formerly believed that they developed in hard-working people in response to exposure of the hands to water or to injury. With this thought in mind, the failure of Heberden's nodes to develop in the injured or paralyzed hand was attributed to lack of use and exposure to injury of that hand. Development of the lesion in the functioning hand was explained as due to increased use. It is readily apparent that the normal hand remaining for use by hemiplegic patients is less exposed to injury than it would have been if the patient had been well and pursuing a normal life. It is now known that hard work or injury is not necessary to explain the occurrence of Heberden's nodes.

Heberden's nodes are typical manifestations of degenerative joint disease. The question arises as to whether the observations of this study have any significance in relation to degenerative joint disease in other parts of the body. Degenerative joint disease usually occurs in middle or later life and is thought to be due to deficient or decreased circulation. Experimental support to this theory has been presented by Goldhaft, Wright and Pemberton.[4] They produce bone proliferation and spur formation in the patellæ of dogs by surrounding this bone with mattress sutures, thus interfering with the circulation to this bone. The prevention of Heberden's nodes described here has been associated with a nerve injury or a lesion in the central nervous system affecting the vasomotor mechanisms in the paralyzed areas. This caused increased blood

FIG. 6.—Mrs. C. C. The left hand before and 15 months after her stroke. Before, there was marked enlargement of all the terminal interphalangeal joints with flexion deformity and deviation of the index and little fingers. After the stroke enlargement of the joints has become less marked, the fingers are decreased in diameter, the skin looks tight and smooth. The fingers show mild flexion deformities.

circulation to bone and resultant osteoporosis.

Demineralization of bone is found in both upper and lower motor-neurone disease, being diffuse in the former and localized in the latter. Both these types of nerve lesions are accompanied by trophic changes in the affected part. Such changes consist of marked alterations in skin temperature, skin color, local circulation, sweating and loss of normal skin markings. There is shrinkage of the limb due to atrophy of subcutaneous tissues, a lead pipe stiffness of the joints, pain on passive motion or pressure and fibrous ankylosis of the joints. The bones themselves show marked osteoporosis and and alteration in bone structure although

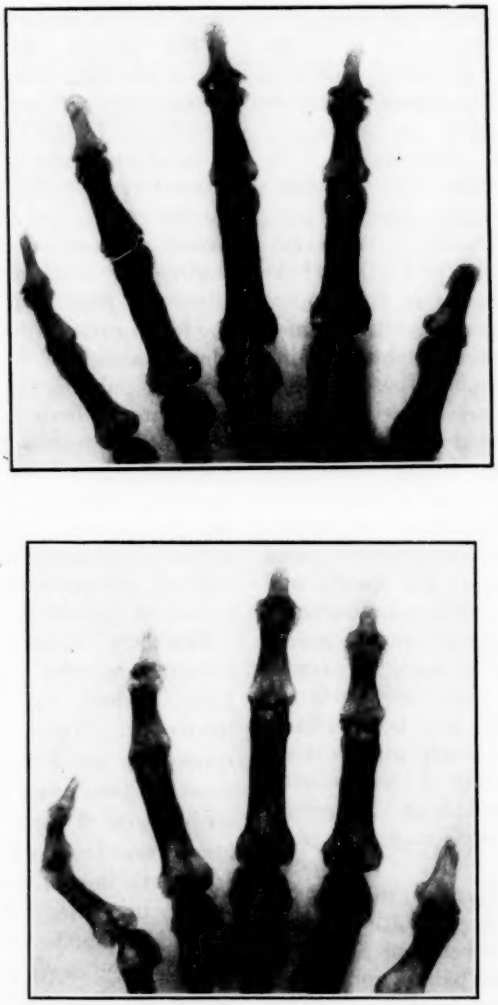

FIG. 7.—Roentgengrams of the left hand before the stroke show enlargement of the proximal ends of all distal phalanges and bulbous swelling of the distal ends of the second phalanges. There is irregular spur formation. The joint surfaces are distorted and uneven, the joint spaces are decreased in width. Roentgengram after the stroke shows marked demineralization of all bones but the size, shape and outline of the bones remain unchanged.

the joint surfaces retain their normal contour and normal joint space is preserved. Osteoporosis occurs very early in response to disuse even in normal limbs where no trophic soft tissue changes occur. The French clinicians who studied "hemiplegia sans arthritis" argued that the demineralization process, whatever its cause, was the factor which interfered with the development of joint disease.[2-4]

The osteoporosis is only one of many responses to vasomotor disorder in a paralyzed limb. de Takats[11] is of the opinion that several apparently different entities in medical literature based on vasomotor disturbance are essentially one and the same phenomenon. He believes, therefore, that Weir Mitchell's causalgia, Sudek's atrophy, Leriche's post-traumatic painful osteoporosis, the peripheral trophoneuroses of Zur Verth and the chronic traumatic edema of Klassen are slightly different manifestations of a fundamental vasomotor irritability due to damage either to peripheral nerves, spinal cord or cerebral centers. He conceives of 3 stages in this morbid process: (*a*) An acute painful phase during which the limb is warm and dry and is sensitive to jarring, air currents and emotional upsets. The subcutaneous and periarticular spaces are edematous, muscles are spastic and oscillometric curves indicate increased circulation. Osteoporosis only appears after 4 or 6 weeks of such hyperemia. (*b*) A second stage when the part is not so warm, and actually may become hard, cyanotic and cold to touch; the joints are stiff and spotty atrophy of bone becomes evident. (*c*) A final stage of general atrophy of skin, muscle and bone with ankylosis.

de Takats is further of the opinion that the offending vasodilatation cannot be due to sympathetic efferent fibers and attributes the peculiar, painful and trophic disorders to an irritation of the so-called nocisensor fibers of Lewis[6] in the posterior root system which are capable of secreting a pain substance at their distal terminations. It is further assumed that such irritation can occur at any of the 3 sensory levels of the nervous system and therefore trophoneuroses and bone atrophy can be found in peripheral nerve injury, poliomyelitis and cerebral thrombosis. This assumption appears to have adequate support in these case presentations where osteoporosis and a concomitant failure of development of Heberden's nodes was found in lesions of nerve trunks, the spinal cord and the gray and white matter of the cerebrum.

The trophic disturbances described above following identifiable injury to the central nervous system led to increased circulation to the limb, demineralization of bone and protection against the development of Heberden's nodes. These conditions must be clearly differentiated from other trophic disturbances of bone leading to neurogenic arthropathy or Charcot's joint. In the latter instance the lesion causes a loss of deep pain sense plus an associated hypotonia of the muscles manipulating the joints. Use of such a hypotonic limb unprotected by pain sense causes serious tissue trauma. It is not clear why such a set of circumstances results in marked bone proliferation, bone destruction and foreign body formation in and about the joint. There is no generalized osteoporosis as invariably occurred in the previous conditions.

Summary. Idiopathic Heberden's nodes (common manifestations of degenerative joint disease) usually involve fingers bilaterally. Nerve injuries of different types may interfere with their development. Three cases of our own are described and 9 cases from the literature are discussed. It was found that peripheral nerve injury, infantile paralysis and stroke prevented the development of Heberden's nodes in affected fingers, though they developed to an advanced degree on the unaffected hand. Such nerve lesions invariably caused osteoporosis which was presumably due to increased blood circulation. Once Heberden's nodes are established, subsequent

cerebral apoplexy causes an apparent regression of the nodes due to shrinkage of soft tissues without alteration in the contour of the bones. Trophic disease preventing the formation of Heberden's nodes must be differentiated from another type of trophic disturbance leading to neurogenic arthropathy or Charcot's joints.

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THE INDICATIONS FOR IRRADIATION OF THE PITUITARY GLAND IN PATIENTS WITH ARTERIAL HYPERTENSION*

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In an earlier publication, Pendergrass, Hodes and Griffith[1] described a bioassay method for detecting an antidiuretic substance in human serum. They considered that this substance originated, in all probability, in the pituitary gland and could therefore be regarded as evidence of increased pituitary activity. Irradiation of the pituitary was carried out in a number of patients who had evidence of such increased activity and, in addition, clinical conditions which might be the result of such increased activity. In this group there were 31 patients with arterial hypertension, 24 of whom had an adequate follow-up. Five patients showed definite improvement in blood pressure and symptoms, 8 showed subjective improvement only, and 11 were not benefited. In the present study a larger number of hypertensive patients were studied, treated and followed for a longer period. Also, certain additional data have been collected with the intention of improving selection of such patients.

The historical aspects of pituitary irradiation have been reviewed previously.[1] Certain additional points, however, should be stressed: (1) In clinical hypertension due to increased activity of the pituitary gland, it is probably the excessive secretion of the pressor hormone that is at fault. Moreover, the pressor hormone is usually, and perhaps always, associated with the antidiuretic hormone. While there is no satisfactory bioassay method for the pressor hormone, one for the antidiuretic hormone is available. For clinical purposes, therefore, it may be assumed that subjects with hypertension who show increased production of antidiuretic hormone also have an excess of pressor hormone. If such is not always the case, it may supply one reason why pituitary irradiation is not always effective. On the other hand, there is ample evidence that antidiuretic hormone may be present without pressor hormone, or, at least, some individuals with positive bioassays for antidiuretic hormone have perfectly normal blood pressure, as witness patients with premenstrual edema.[1] (2) Antidiuretic substance may be present in the serum of a man or animal without the subject showing antidiuresis after water ingestion. The explanation for this is uncertain, but it may depend upon the development of a refractory state or the production of an opposing diuretic substance, as previously considered by Griffith, Kimbrough, Corbit and Roberts.[2] (3) Antidiuretic substance may originate elsewhere than in the pituitary, as described by Walker.[3] However, when the antidiuretic substance disappears from the serum of patients 1 to 2 months after pituitary irradiation, it appears likely that it can be regarded as being of pituitary origin.

* Except for an occasional revision, this material was presented in the Scientific Exhibit, September 1944, of the American Roentgen Ray Society and the Radiological Society of North America, Inc., where it received the first award.

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Material and Methods of Study. All patients treated were adults suffering from arterial hypertension. In all, 142 subjects received roentgen therapy, 62 males and 80 females. However, adequate follow-up was secured on only 93 patients, and only these appear in the charts. The follow-up period ranged from 3 to 56 months, averaging 16 months. The few patients with the short follow-up period of only a few months invariably represented very ill patients whose results following therapy were poor and who could not return for later studies. If all patients with a follow-up period of less than 6 months had been eliminated, however, the results of therapy would have appeared better than they were. As for age, the middle decades are best represented, 47% of the women being between the ages of 40 and 50, and 44% of the men between 50 and 60

Methods of Study. 1. Bioassay for antidiuretic hormone in serum. This was initially positive in all cases that were treated, and was the primary basis for selection. The technique is unchanged from that previously described.[1] Bioassays were repeated at monthly intervals for 3 months after irradiation and thereafter, when possible, every 3 to 6 months.

2. Roentgenograms of the pituitary fossa, in most cases.

3. In some cases, bioassay for gonadotropic hormone, presumably originating in the anterior lobe of the pituitary, at the level of 330 m.u. per 100 cc. of serum, by the mouse method of Rakoff.[4]

4. Measurement of renal function, in most cases, by the plasma creatinine method of Steinitz and Turkand.[5]

5. Measurement of cutaneous lymphatic flow, in most cases, by the patent blue method of McMaster.[6] This was selected because in the rat increased flow can be demonstrated by this method following the ingestion of water and the administration of an antidiuretic dose of pitressin.

6. Measurement of cutaneous capillary reactivity by direct observation under the capillary microscope, by the method described by Griffith, Roberts and Corbit.[7]

7. Roentgen study of the urinary tract with the aid of diodrast or neo-iopax. This study is suggested because one can obtain additional evidence about individual kidney function. Likewise silent stones and unsuspected kidney lesions of significance occasionally are found.

Technique of Roentgen Therapy. Since the previous publication, the tendency has been to use larger doses. While it will be seen in Table 4 that doses have been used varying from less than 1000 to 2000 R in air, and some good results have been achieved with low doses, the higher dose is preferred and 49 of the 93 cases here reported were treated by it. The factors used at the present time are: kilovoltage, 200; milliamperage, 15; filtration, 0.5 mm. Cu plus 1 mm. Al added; half-value layer, 1 mm. Cu; type of machine, constant potential, oil-cooled; roentgens per min., 68.7; target-skin distance, 50 cm.; number of portals, 2; size of portals, 9 cm. diameter; site of portals, lateral temporal, directed at the pituitary fossa; daily exposure, 50 to 200 roentgens to 1 portal; time of administration, 12 days; total exposure, 2 × 1000 roentgens in air.

Results. It will be seen in Tables 1, 2 and 3 that slightly less than half the patients treated had a significant lowering of blood pressure, and slightly more than half showed appreciable clinical improvement. There is a good correlation between treatment and change in systolic and diastolic blood pressure, and between change in blood pressure and clinical improvement. The material is further broken up into 9 groups as shown in Table 4, where the best results are indicated in the columns on the left and the worst in the right-hand columns. However, it should be noted that in the ninth group only can one be certain that no benefit was obtained, and it is this group that deserves further analysis so that such cases in the future would be either not treated, or treated differently. It is apparent from the upper part of the table that the principal finding, associated with failure of therapy, is failure of the bioassay for antidiuretic hormone to become negative. This might suggest incorrect diagnosis except that it occurs less frequently when larger doses of irradiation are given, and, recently, we have undertaken a second similar course of irradiation within 3 months of the first course, if,

TABLE 1.—EFFECT OF PITUITARY IRRADIATION UPON BLOOD PRESSURE

(*a*) *Systolic Blood Pressures Before Irradiation*

		Over 250	200–250	170–200	150–170	Below 150
Systolic Blood Pressure After Irradiation	Over 250	4				
	200–250	*1*	24			
	170–200	..	*11*	16		
	150–170	..	(7)	*8*	5	
	Below 150	..	(11)	(6)	5	

(*b*) *Diastolic Blood Pressure Before Irradiation*

		Over 140	120–140	110–120	100–110	Below 100
Diastolic Blood Pressure After Irradiation	Over 140	14				
	120–140	*1*	20			
	110–120	..	*6*	11		
	100–110	(1)	(5)	*6*	6	
	Below 100	..	(10)	(9)	(4)	

Numbers refer to number of patients. Those in parentheses are thought to show good results and those italicized fair results.

TABLE 2.—CORRELATION BETWEEN CHANGE IN SYSTOLIC AND IN DIASTOLIC BLOOD PRESSURE FOLLOWING PITUITARY IRRADIATION

		Systolic Blood Pressure Change		
		Good	Fair	No change
Diastolic Blood Pressure Change	Good	21	7	1
	Fair	1	12	
	No change	2	1	48

TABLE 3.—CORRELATION BETWEEN CHANGE IN SYSTOLIC BLOOD PRESSURE AND CLINICAL IMPROVEMENT

		Systolic Blood Pressure Change		
		Good	Fair	No change
Clinical Improvement	Definitely good	17	7	6
	Probably good	6	10	12
	No change	1	3	31

TABLE 4.—FACTORS INFLUENCING THE RESPONSE TO PITUITARY IRRADIATION IN HYPERTENSION

Group No.:	Clinically and B.P. good 1	Clinically good, B.P. fair 2	Clinically probably good, B.P. good 3	Clinically probably good, B.P. fair 4	Clinically good, B.P. no change 5	Clinically no change, B.P. good 6	Clinically probably good, B.P. no change 7	Clinically no change, B.P. fair 8	Clinically and B.P. no change 9
After treatment, bioassay antidiuretic hormone became									
Negative	17	7	5	10	6	1	7	2	10
remained Positive	..	..	1	..	..	..	5	1	21
Before treatment, bioassay for gonadotropic hormone at 330 m.u.:									
Negative	9	5	2	7	2	..	6	1	14
Positive	..	..	..	1	..	..	..	1	5
Before treatment renal function:									
Normal	10	6	2	6	2	..	5	2	16
Abnormal	..	1	..	1	..	..	2	1	7
Before treatment cutaneous capillary reactions:									
Normal	11	3	3	7	1	1	3	2	16
Abnormal	4	1	..	3	1	..	4	..	9
Before treatment cutaneous lymphatic flow:									
Normal	6	4	2	6	..	..	5	..	2
Abnormal	6	1	3	4	1	1	6	3	5
Roentgen dosage:									
2000 R or more	10	6	2	7	1	..	5	1	17
1500–1990 R	4	..	2	1	3	1	3	2	7
1000–1490 R	3	..	1	..	2	..	3	..	4
Under 1000 R	..	1	1	2	..	..	1	..	3
"Selected" cases:									
(*a*) Renal function normal, gonadotropic hormone absent	8	4	2	5	2	..	3	1	10
(*b*) As (*a*) but only cases where AD became negative after treatment	8	4	1	4	2	..	1	..	4

after that interval, the test for antidiuretic hormone remains positive.

It is apparent also, in Table 4, that persons with renal failure, or with a high titer for gonadotropic hormone in the serum, are apt to do badly after irradiation. While there appears to be a tendency for patients with normal capillary reactions to do better than those with abnormal reactions, and for those with increased cutaneous lymphatic flow to do better than those with normal flow, the tendency is not sufficiently marked to be useful in selection. The last section of Table 4, under (*a*), shows that by selection of patients on the basis of a positive bioassay for antidiuretic hormone, normal renal function, and a normally absent bioassay for gonadotropic hormone, one should reduce the group of complete failures (Group 9) to 38%, and if one further eliminates the group given an insufficient amount of irradiation (which is the best explanation for the group where the bioassay does not become negative) the group of complete failures (*b*) would appear to be only 16%, based upon the small series that remain.

The pituitary fossa was enlarged in 7 of 28 cases. This did not seem to bear any relationship to the result of therapy. Where the fossa was enlarged, a pituitary tumor was suspected and in certain instances, the roentgen ray dosage was increased.

Complications of therapy, except for the routine hair loss, did not occur in subjects who did not have papilledema. However, 5 patients with papilledema were treated, and 3 of the 5 showed a fairly characteristic reaction. About 6 hours after the first treatment a severe headache would develop, and this, in a few hours, would be followed by stupor, or delirium, finally coma and convulsions, from which the patient could be partially aroused by lumbar puncture with withdrawal of fluid to relieve pressure, and by hypertonic glucose given intravenously. The following morning the patient would appear to be quite normal and usually had no clear recollection of the previous evening. Irradiation was continued, cautiously, and subsequent reactions did not occur. This crisis is thought to be due to a choroid plexus hyperemia, with increased formation of cerebrospinal fluid. It is, therefore, important that all patients who present papilledema be hospitalized during treatment. Since none of our patients with papilledema has shown a complete recovery after roentgen therapy, its routine use under these circumstances is not recommended; however, 1 woman did have a remission in which her papilledema subsided, the symptoms cleared and, although her blood pressure did not reach normal, she was much improved and able to do the work for her large family for 5 years, or until 6 months before her death in uremia.

Following irradiation, there has been no evidence of hypopituitarism. Electroencephalographic studies were carried out in 12 patients who had received a total roentgen dosage ranging from 430 to 4760 R. Those patients given more than 2000 R had received more than 1 course of roentgen therapy but no evidence of damage to brain tissue was found.

Relapses occurred 13 times in 10 patients, varying from 6 months to 4 years after irradiation, averaging 18 months. Eight patients were given 2 courses of treatment, and 1 patient was given 3.

Summary and Conclusions. Pituitary irradiation as a treatment for high blood pressure was given to 142 patients without untoward effect, although 3 persons with papilledema showed severe acute but transient reactions indicating increased intracranial pressure. Only 93 patients were adequately followed for a period varying from 3 to 56 months, averaging 16 months. About half of these persons showed improvement in blood pressure and clinical condition. All of these individuals were selected on the basis of a positive test for antidiuretic hormone in the serum. From a consideration of other studies and of the results of varying roentgen dosage, it is concluded that the chance of benefit from radiation therapy to the

pituitary in hypertension should be at least 75% if cases are selected according to the following criteria: (1) Positive bioassay for antidiuretic hormone in serum. (2) A roentgen dosage of 1000 R delivered into the hypophysis (2000 R in air), to be repeated in 3 months if the test for antidiuretic hormone in serum has not become negative in that time. (3) A negative bioassay for gonadotropic hormone in serum, at the level of 330 m.u. per 100 cc. of serum. (4) A normal renal function as shown by a plasma creatinine, by the method of Steinitz and Turkand, of 1 mg. % or less. (Presumably a test of urea clearance would be equally satisfactory.) (5) Good clearance of injected dye from each kidney as shown by urography.

Case Reports. Case 1. Patient, an apparently healthy white male, was first seen Feb. 26, 1941. He was 23 years old, and about a year previously his school physician had discovered his systolic blood pressure was 175. Several similar readings had been obtained. Physical examination was essentially negative except for the blood pressure which, after a week in bed, was 165/100. The usual laboratory studies, including tests for kidney function and urogram, were entirely negative. Bioassay for antidiuretic hormone was positive on 2 occasions. Accordingly, he was given a course of irradiation to the pituitary from September 3 to 11: 5 cm. portals to temporal areas; 710 R (in air) to left temporal portal; 790 R (in air) to right temporal portal. Thereafter, he returned to school, and a report from his physician, October 20, stated that his blood pressure was no more than 150/95. On December 26, bioassay for antidiuretic hormone was entirely negative. Thereafter, blood pressure remained about 140/90, and in March 1944 he entered the army, with a systolic blood pressure recorded as 135. He had no difficulty in carrying out fairly rigorous duties for the next 2 years, and blood pressure was found within normal limits whenever tested. His own physician found a blood pressure of 126/80 shortly after his discharge, in May 1946. The army records included a description of an examination in 1940 when he had applied for reserve officers training in connection with his college program and had been rejected with a blood pressure of 175. At the separation center the medical officer insisted that some error had been made in confusing the records of 2 different men.

Case 2. Patient, a white male 37 years of age, was first seen March 3, 1941. For 1½ years his family physician had found his blood pressure ranging about 180/120. However, after several days in the hospital, it fell to 155/100. Physical examination was otherwise essentially negative, and the usual laboratory studies were normal. Bioassay for antidiuretic hormone, however, was positive, and patient was given a course of pituitary irradiation from March 7 to 15: 5 cm. portals to temporal areas; 695 R (in air) to left temporal portal; 805 R (in air) to right temporal portal.

Bioassays for antidiuretic hormone were subsequently negative on April 14, May 16, June 27, September 8 (1941), and Feb. 6, 1942, and on the same dates blood pressure was 140/90, 138/88, 136/90, 130/82, and 138/90. His own physician has measured his blood pressure half a dozen times a year since, and states that it has never been over 138/90, and is usually 10 to 12 points lower.

Case 3. Patient, a white male 47 years of age, was first seen Oct. 24, 1945. He complained of some palpitation, and dyspnea during coitus. Hypertension had been discovered 3 months previously, and 9 months previously he had successfully passed a physical examination for aviation pilot. Physical examination, however, in our laboratory revealed a blood pressure of 200/128 and cardiac enlargement. Bioassay for antidiuretic hormone was positive and plasma creatinine was 1.3 mg. % (top normal by this method 1 mg. %). Roentgen ray examination of pituitary fossa, gall bladder, urogram was essentially normal. Electrocardiogram revealed sinus rhythm with ventricular extrasystoles, broad and enlarged P waves, and a slurred QRS complex with left axis deviation. Orthodiagram showed cardiac enlargement. Urinary specific gravity was 1.016 without attempt at concentration. Although it was recognized that patients with renal damage usually do badly after pituitary irradiation, the only evidence suggesting renal involvement was the slightly elevated plasma creatinine. Accordingly, a course of pituitary irradiation was given November 26 to December 7: 7 cm. portals

to temporal areas; 1050 R (in air) to left temporal portal; 1000 R (in air) to right temporal portal.

On Jan. 8, 1946, patient stated he felt much better, had no more palpitation and very little dyspnea during coitus. Blood pressure was 182/128, bioassay for antidiuretic hormone was negative but plasma creatinine was 1.7 mg. %. On February 19 patient still felt well, blood pressure was 186/104, bioassay for antidiuretic hormone was negative, and plasma creatinine was 1.8 mg. %. On April 17 he was definitely worse, with nausea and nocturnal dyspnea, blood pressure 186/118 positive bioassay for antidiuretic hormone, and plasma creatinine of 2.2 mg. %. On June 20 he was hospitalized with a blood pressure of 196/118, negative bioassay for antidiuretic hormone, and plasma creatine of 3.9 mg. %. Subsequent course was downhill, and he died with typical uremia on July 7. At necropsy, the heart was found to be markedly enlarged, with left ventricular hypertrophy. There was passive congestion of the liver and spleen and diffuse pulmonary edema. The kidneys were described as being of the granular hypertensive type but were not markedly contracted and the pathologist felt there was less cortical atrophy than might have been anticipated. The urinary tract showed no evidence of infection or congenital anomaly.

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FURTHER STUDIES ON THE CORRELATION OF CHEMICAL STRUCTURE AND ANTITHYROID EFFECT

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The early clinical experiences with thiouracil indicated that serious toxic reactions sometimes occurred and this prompted an investigation, beginning in 1943, of other antithyroid compounds. It was hoped that the incidence and severity of the toxic reactions might be reduced by obtaining compounds with stronger antithyroid action, thereby exposing the body tissues to a smaller concentration of the drug, or by finding substances which by their difference in chemical structure were less toxic than thiouracil even though their concentration in the body might be greater. During the course of these studies there have appeared reports of similar studies by McGinty and colleagues,[4,8,9] and Astwood's investigations[1] have been greatly extended.[2] In addition to the compounds which we have reported upon[11] others have been investigated.[5,6,7]

Plan of Study. In his early studies Astwood[1] found that most of the compounds with pronounced goitrogenic effect were derivatives of thiourea or aniline. Most of the compounds* which we have studied have been of these types, there being numerous alterations in the structure of the molecules. In addition, a few organic sulfur compounds were tested. The basic structure of some of the more interesting compounds which have been tested in this and in previous studies are shown structurally in Figure 1.

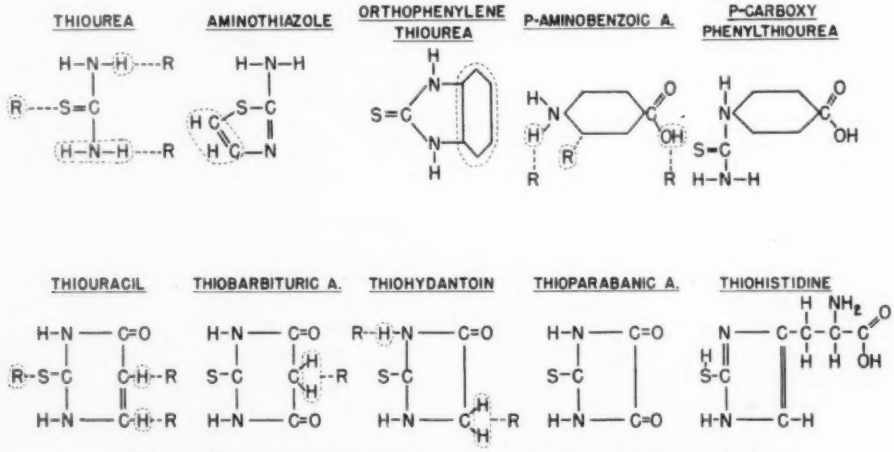

Fig. 1.—Basic structure of some of the interesting compounds studied.

* We are very grateful to Mr. W. A. Lott and the late Dr. George A. Harrop and their associates at the E. R. Squibb & Co., New Brunswick, New Jersey, for supplying us with most of the compounds tested and for aid in many other ways. Dr. R. A. Harte of the Arlington Chemical Company, Yonkers, New York, kindly supplied a few of the chemicals which were tested. Dr. R. O. Roblin, of the American Cyanamid Company, Stamford, Connecticut, supplied the 6-α-ethylpropyl-2-thiouracil.

It was especially desirable to test the antithyroid effect of iodinated thioureas and iodinated aminobenzenes. It was hoped that the thyroid gland's usual affinity for iodine would prevail in this instance and as a result of the high concentration of the antithyroid agent it would be unnecessary to expose the remainder of the body to large amounts of the compound. . At the same time there was full realization of the fact that the usual mechanisms of fixation of iodine in the thyroid[10] might be antagonized by the thiourea or aminobenzene portion of the molecule. One would not expect a very striking effect in normal animals even though the compound might be practical in thyrotoxic subjects, because normal thyroid glands do not have as much avidity for iodine. Nevertheless, it was regarded as being worthy of a trial. It has not been possible for us to obtain an iodinated thiourea compound without a radical attached to the sulfur; many thioureas lose their antithyroid effect when the sulfur atom bears a substituent.

The detailed methods of study have been described.[11] The rats used ordinarily weighed about 40 gm. at the beginning of the experiment. Their healthy littermates gained, on the average, 4 gm. per day. The animals were given the test compounds in the drinking water, great care being taken to avoid wasting. After ingesting the solution for 14 days, the rats were killed and the thyroid gland was quickly removed and weighed.

Since thiohistidine is a natural constituent of the blood, it was especially desirable to determine whether it possessed any antithyroid effect. Although it had been previously studied in rats,[11] and had been found to be without antithyroid effect, the quantity available was small. As an additional test animal the chick was used to study this compound. To 10 cc. of 0.2% agar in saline was added 100 mg. of thiohistidine. Each of 3 chicks was injected, subcutaneously, with 0.1 cc. of the mixture twice daily for 14 days, beginning when the chicks were 2 days old. The animals were then killed, along with the controls, and their thyroid glands were quickly removed and weighed.

Results. *Thiouracils.* The most potent antithyroid compounds found were thiouracils with short-chain hydrocarbons in the 6-position. Of these, the *n*-propyl derivative was the most active one, and it was also the most effective one found by Astwood, Bissell and Hughes.[2] Cyclopropylthiouracil and isobutylthiouracil were almost as active as was *n*-propylthiouracil. The effectiveness of *n*-butylthiouracil and amylthiouracil was much less marked, but all of these were distinctly more active than was thiouracil (Fig. 2).

Of the compounds tested with substituents in the 5-position, ethylthiouracil was the only one that was more active than thiouracil. The activity of the 5-ethylthiouracil was abolished when an ethyl radical was attached to the sulfur, as in 2-ethylmercapto-5-ethyluracil (Table 1). When an amino group was in the 5-position the activity was lost.

The toxicity of all of the thiouracils was relatively low. There was no significant inhibition of growth. Moreover, microscopic examination of the liver, spleen, pancreas, kidneys, adrenals and gonads revealed that these organs were normal in appearance.

Other Thioureas. None of this group of compounds tested possessed significant activity, with the exception of 2-aminothiazol (Table 2). The latter compound, although many times less active than thiouracil, produced a distinct effect when large doses were used. Several thioureas were fairly toxic, especially bis-(methylpiperidine)thiourea, 1, 4-diazabicyclo(2,2,-1)heptane and bis-(2-hendecyl-2-imidazoline-1-ylethyl)thiourea.

The chicks, treated with thiohistidine, experienced no thyroid enlargement.

Anilides. Several anilides with structures similar to p-aminobenzoic acid were studied (Table 2). The activity of each of these compounds tended to be relatively slight (Table 2), even in large doses. The

TABLE 1—RELATION OF CHEMICAL STRUCTURE TO GOITROGENESIS

Compound	Formula	Concentration in drinking water (%)	Total amount ingested (mg./100 gm.)	Average weight at death (gm.)	Weight of thyroid (mg./100 gm.) Range	Weight of thyroid (mg./100 gm.) Average
THIOURACILS						
2-Thiouracil	$NHCSNHCOCHCH$	0.001	2.4	81	6.3– 7.1	6.7
		0.002	4.6	107	9.2–10.5	9.9
		0.004	9.4	86	5.5–10.3	8.4
		0.005	14.4	151	7.4–10.8	8.7
		0.005	12.1	80	5.3– 6.2	5.8
		0.006	12.9	84	8.1–10.2	8.8
		0.007	17.8	76	8.7–12.5	10.2
		0.008	21.2	91	8.3–10.0	9.0
		0.01	23.8	159	9.1–14.2	11.4
		0.01	23.6	79	11.6–16.4	13.1
		0.01	20.1	84	5.4–11.1	9.1
		0.01	20.4	71	7.1–10.5	8.5
		0.01	24.3	89	4.8–13.2	8.2
		0.01	26.8	104	8.3–11.7	9.7
		0.02	51.6	85	6.3–11.0	8.2
		0.02	35.9	76	6.1– 8.0	7.0
		0.02	46.8	113	14.2–21.1	17.6
		0.04	77.0	88	9.9–19.5	14.1
		0.04	81.0	69	4.6–15.1	9.8
		0.04	86.0	113	13.8–26.6	18.2
		0.05	107.5	70	13.0–13.3	13.2
2-Thio-5-ethyluracil	$NHCSNHCOC(C_2H_5)CH$	0.001	2.2	115	5.8– 8.1	7.2
		0.003	6.2	107	8.3–10.7	9.6
		0.005	11.5	118	16.1–20.4	17.2
		0.007	14.8	110	9.7–21.4	16.3
		0.01	16.5	121	15.2–21.1	18.6
		0.02	42.3	123	15.1–18.2	16.1
2-Thio-5-aminouracil	$HCSNHCOC(NH_2)CHN$	0.01	22.9	98	6.0– 8.9	7.0
2-Thio-5-phenylacetylaminouracil	$NHCSNHCOC(NHCOCH_2C_6H_5)CH$	0.01	34.3	92	6.0– 6.2	6.1
2-Ethylmercapto-5-methyluracil	$NCS(C_2H_5)NHCOC(CH_3)CH$	0.1	235.0	85	3.6–11.7	7.4
2-Ethylmercapto-5-ethyluracil	$NCS(C_2H_5)NHCOC(C_2H_5)CH$	0.01	21.2	131	7.3– 8.3	7.8
2-Thiouracil-4-aldehyde	$NHCSNHCOCHCCHOH_2O$	0.0001	0.27	95	6.4– 8.2	7.3
		0.0005	1.21	99	6.0– 8.0	6.8
		0.001	2.78	126	5.8– 8.4	7.3
Diethylacetal-2-thio-4-uracil aldehyde	$NHCSNHCOCHCCH(OC_2H_5)_2$	0.0001	0.21	97	6.0– 9.6	7.7
		0.0005	1.27	118	5.2–10.7	7.6
		0.001	2.7	131	10.0–14.1	12.4
2-Thio-6-*n*-propyluracil	$NHCSNHCOCHCC_3H_7$	0.0001	0.23	115	7.5–10.6	8.4
		0.0003	0.75	120	8.9–10.4	9.7
		0.0005	1.2	120	15.2–16.5	15.9
		0.001	2.4	103	7.9–36.4	20.6
		0.003	6.7	132	28.8–35.6	32.7
		0.005	11.9	115	36.2–40.6	37.7
2-Thio-6-cyclopropyluracil	$NHCSNHCOCHCCHCH_2CH_2$	0.0001	0.3	129	5.3– 6.2	5.7
		0.0003	0.8	120	6.6– 8.9	7.8
		0.0004	0.78	125	8.3– 9.9	8.9

		0.005	11.9	115	36.2–40.6	37.7
2-Thio-6-cyclopropyluracil	$NHCSNHCOCHCCHCH_2CH_2$	0.0001	0.3	129	5.3– 6.2	5.7
		0.0003	0.8	120	6.6– 8.9	7.8
		0.0004	0.78	125	8.3– 9.9	8.9
		0.0005	1.24	119	10.0–12.1	10.8
		0.001	2.4	136	9.4–14.8	11.6
		0.003	8.3	131	32.4–34.4	33.3
		0.005	9.4	84	26.1–35.6	30.6
2-Thio-6-*n*-butyluracil	$NHCSNHCOCHCC_4H_9$	0.0003	0.80	109	7.1– 8.8	7.9
		0.0005	1.2	127	5.8– 8.4	6.9
		0.0007	1.6	107	8.3– 8.6	8.4
		0.001	2.5	110	8.5–10.9	9.8
		0.003	7.3	113	8.4–14.5	11.5
		0.005	10.7	84	18.6–25.2	22.2
		0.007	13.7	156	10.2–13.1	11.7
		0.01	17.8	117	10.6–17.1	14.5
2-Thio-6-isobutyluracil	$NHCSNHCOCHCCH_2CH(CH_3)_2$	0.0001	0.35	74	6.3–12.3	8.9
		0.0001	0.30	72	6.4– 9.4	7.8*
		0.0002	0.55	66	6.5–14.0	11.0
		0.0002	0.60	65	8.8–11.9	10.4*
		0.0003	0.65	70	7.2–10.6	9.0
		0.0003	0.69	81	6.6– 9.8	8.2*
		0.0003	0.90	71	9.0–12.0	10.7
		0.0003	0.95	67	5.9– 9.9	8.4*
		0.0003	0.62	101	7.2– 7.9	7.5
		0.0003	0.70	99	5.8– 7.5	6.7*
		0.0004	0.96	62	6.9–10.3	8.9
		0.0004	0.85	74	7.7–11.3	9.8
		0.0005	0.31	129	10.8–13.3	12.3
		0.0005	1.27	118	10.1–16.5	12.8*
		0.001	2.5	108	13.4–15.3	14.0*
		0.001	2.3	90	15.3–19.9	17.5
		0.002	7.3	91	25.6–29.6	27.9*
		0.005	14.2	57	13.0–16.0	14.1
		0.005	11.1	74	32.1–41.4	36.4
		0.005	10.4	83	16.6–22.7	18.7
		0.005	11.2	69	39.7–47.5	43.7*
		0.01	19.6	113	23.8–33.7	27.7*
		0.01	21.6	89	30.8–40.1	35.5
2-Thio-6-amylthiouracil	$NHCSNHCOCHCC_5H_{11}$	0.0005	1.3	128	6.2– 8.0	7.2
		0.0007	1.8	120	8.5– 8.9	8.6
		0.0007	1.6	118	6.7– 8.8	7.5
		0.001	3.0	119	7.0– 8.7	7.5
		0.001	2.0	108	7.3–14.5	10.3
		0.002	4.6	120	7.0– 9.1	8.1
		0.002	4.8	112	7.3–10.7	9.4
		0.003	6.0	112	5.5–12.4	8.8
		0.005	11.1	114	9.5–10.2	9.9
		0.005	11.4	65	11.1–14.4	12.9
		0.005	11.9	122	11.4–16.1	13.6
		0.01	21.0	104	12.7–28.4	21.0
		0.01	16.5	87	13.7–24.3	19.5
2-Thio-6-α-ethylpropyluracil	$NHCSNHCOCHCCH(C_2H_5)C_2H_5$	0.0003	0.49	102	7.8–10.3	8.9
		0.001	1.9	103	8.7–10.0	9.2

* These tests were conducted on a compound which was first thought to be isoamylthiouracil. However, it proved to be isobutylthiouracil. The net result was a comparison of isobutylthiouracil obtained from two different pharmaceutical companies.

toxicity of diaminobenzophenone and p-aminocinnamic acid was fairly great.

Iodinated Compounds. The iodinated compounds demonstrated little or no antithyroid effect. Moreover, several of these compounds were relatively toxic. Three groups of rats receiving 0.5% 2-amino-3,5-diiodobenzoic acid succumbed after treatment for 4 or 5 days. The 4-amino-3,5-diiodobenzoic acid was less toxic and the monoiodinated homologue was much less toxic.

of the various antithyroid compounds. In some instances the substances are relatively much more effective in rats than in humans and sometimes the reverse is true. Moreover, there are occasionally marked variations in the same species. That such discrepancies should exist is indicated by the variations in the absorption, distribution and excretion of the compounds in rats and man.[12]

In some respects there are striking cor-

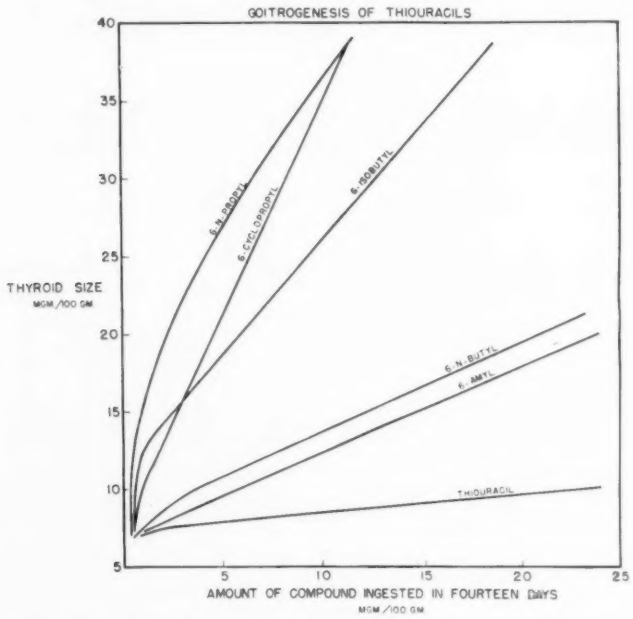

FIG. 2.—Note that these derivatives of thiouracil are much more goitrogenic, or antithyroidal, than is the parent compound. The size of the thyroid gland and the amount of drug ingested are expressed in terms of body weight of the animal.

Combined Treatment With Para-aminobenzoic Acid and Thiouracil. It can be seen in Table 2 that when p-aminobenzoic acid is given simultaneously with thiouracil the amount of goitrogenesis equals, or perhaps exceeds, an additive effect of the 2 compounds.

Discussion. In the clinical application of the results presented in this paper and in previous ones it must be borne in mind that the assays on rats are but coarse approximations of the relative activities

relations of chemical structure to antithyroid effect. However, in many instances slight changes in molecular structure lead to very unpredictable results, sometimes markedly increasing the activity and at other times reducing it.

On the basis of the observations of various investigators, especially Astwood's, many generalizations may be made in correlating antithyroid activity with chemical structure. The activity of thiouracil was distinctly *decreased*, or in some in-

TABLE 2—RELATION OF CHEMICAL STRUCTURE TO GOITROGENESIS

Concentration	Total	Average	Weight of thyroid

TABLE 2—RELATION OF CHEMICAL STRUCTURE TO GOITROGENESIS

Compound	Formula	Concentration in drinking water (%)	Total amount ingested (mg./100 gm.)	Average weight at death (gm.)	Weight of thyroid (mg./100 gm.) Range	Weight of thyroid (mg./100 gm.) Average
OTHER THIOUREAS						
2-Aminothiazol	$NH_2CSCHCHN$	0.1	193.0	86	7.1–11.2	8.5
		0.3	464.0	80	18.5–34.2	25.6
Bis-(methylpiperidine)thiourea	$C_5H_{10}NCH_2NHCSNHCH_2NC_5H_{10}$	0.0005	1.5	87	6.6– 8.2	7.4
		0.001	3.0	82	7.3– 8.9	8.1
3,4-Dihydro-6-thio-1-thiapyrano(3,4-e)pyrimidine-6,8-dione	$C_5H_6SNHCSNHCO$	0.01	234.0	105	3.8– 8.2	5.7
2-Thiol-4-hydroxy-quinazoline	$NCSHNCOHC_6H_4$	0.01	21.0	109	6.9– 7.1	7.0
p-Carboxyphenylthiourea	$NH_2CSNHC_6H_4COOH$	0.01	29.0	108	6.6– 8.2	7.5
2,6-Dimethyl-4-thiourea pyrimidone	$NC(CH_3)NC(NHCSNH_2)CHC(CH_3)$	0.1	280.0	83	6.3– 7.5	6.9
Bis-(2-heptadecyl-2-imidazoline-1-ylethyl)thiourea	$(C(C_{17}H_{27})NCH_2CH_2NCH_2CH_2NH)_2CS$	0.01	27.4	86	7.1– 8.5	7.6
Bis-(2-hendecyl-2-imidazoline-1-ylethyl)thiourea	$(C(C_{11}H_{23})NCH_2CH_2NCH_2CH_2NH)_2CS$	0.01	28.6	51	...	4.3
1,4-Diazabicyclo(2,2,1)heptane-7-thione	$NCH_2CH_2NCHCH_2CS$	0.01	29.2	64	...	4.6
5-Phenyl-2-thiobarbituric acid	$NHCSNHCOC(C_6H_5)CO$	0.1	299.0	78	5.6– 8.8	7.3
ANILIDES						
p-Aminobenzoic acid	$NH_2C_6H_4COOH$	1.0	2460.0	63	9.3–17.2	12.4
p-Aminohippuric acid	$NH_2C_6H_4CONHCH_2COOH$	1.0	2920.0	57	4.1–12.6	9.7
p-Nitrohippuric acid	$NO_2C_6H_4CONHCH_2COOH$	1.0	2165.0	63	6.9– 9.0	7.7
p-Aminocinnamic acid	$NH_2C_6H_4CHCHCOOH$	0.1	198.0	92	4.4– 8.7	6.5
		0.3	657.0	154	6.5– 8.8	7.3
		0.5	1695.0	28	34.0–58.0	47.5
		0.5	1042.0	140	3.3– 8.2	6.3
4,4'Diaminobenzophenone	$NH_2C_6H_4COC_6H_4NH_2$	0.1	215.0	121	6.7– 7.6	7.1
		0.3	625.0	72	6.1– 9.9	8.0
		0.5	695.0	49	10.5–17.4	14.3
		0.5	1127.0	51	7.8–20.5	14.1
IODINATED COMPOUNDS						
2-Ethylmercapto-5-iodo-6-oxypyrimidine	$NCS(C_2H_5)NHCOCICH$	0.1	312.0	75	7.2–18.4	11.8
p-Amino-3-iodobenzoic acid	$NH_2IC_6H_3COOH$	0.1	259.0	88	8.3– 8.3	8.3
		0.1	620.0	95	...	9.7
		0.1	271.0	86	4.7– 7.0	6.1
		0.5	1410.0	83	5.7– 8.1	6.8
p-Amino-3,5-diiodobenzoic acid	$NH_2I_2C_6H_2COOH$	0.1	266.0	89	...	11.6
		0.1	624.0	97	...	8.8
		0.1	320.0	73	6.5– 9.7	8.3
p-Amino-3,5-diiodobenzoic acid	$NH_2I_2C_6H_2COOH$	0.5	1810.0	64	6.9–13.1	9.6
3,5-Diiodosulfanilamide	$NH_2C_6H_2I_2SO_2NH_2$	0.1	283.0	117	7.5–10.0	8.4
		0.3	790.0	100	5.7– 9.4	7.8
		0.5	1270.0	114	6.1– 7.2	6.5
1-Amino-2-iodobenzene-4-sulfonamide	$NH_2C_6H_3ISO_2NH_2$	0.5	1100.0	118	6.4– 8.9	7.7
3,5-Diiodo-4-hydroxyphenylacetic acid	$I_2OHC_6H_2CH_2COOH$	0.3	715.0	98	5.6– 6.7	6.1
PARAMINOBENZOIC ACID + THIOURACIL						
2-Thiouracil	$NHCSNHCOCHCH$	0.01	29.6	81	9.1–10.2	9.9
p-Aminobenzoic acid	$NH_2C_6H_4COOH$	0.5	1330.0	77	3.9– 8.9	7.1
2-Thiouracil + p-aminobenzoic acid	.....	0.01	25.5	75	10.7–19.0	14.4
		0.5	1295.0			
2-Thiouracil	.....	0.005	11.7	116	7.2– 8.9	7.8
p-Aminobenzoic acid	.....	0.5	1440.0	139	7.6– 8.2	8.0
2-Thiouracil + p-aminobenzoic acid	.....	0.005	12.9	142	9.9–10.4	10.1
		0.5	1290.0			

stances lost, by any of the following changes in structure:

1. Saturation of the double bond, as in dihydrothiouracil.
2. Addition of methyl or ethyl substituents on the nitrogen atoms.
3. The presence of methyl or butyl radicals in the 5-position.
4. The presence of substituents on the sulfur atom.
5. Additions in the 5- or 6-position of amino, carboxy, carbethoxy or cyano groups.

On the other hand the activity of thiouracil was *increased* by:

1. The addition of short-chain hydrocarbons or the benzyl radical to the 6-position.
2. The presence of an ethyl or propyl group on the 5-position. Substituents in the 5 *and* 6 positions (in 1 compound) showed no potentiation of effect.

The activity of thiourea is augmented by the addition of certain short-chain hydrocarbons, as in diethylthiourea and tetramethylthiourea. On the other hand, in general the activity is greatly decreased or lost by:

1. The addition of long-chain alkyl or aryl radicals.
2. The presence of amino, imino or carbonyl radicals attached to 1 or both nitrogen atoms.
3. Substitutions on the sulfur atom.
4. Conjugation of 2 thiourea molecules.

It is of interest to point out that mere closure of the ring as in phenylthiourea, giving o-phenylene thiourea, greatly increases the antithyroid effect.[4] In a similar manner the activity of ethylthiourea is increased when the ring is closed to form 2-imidazoline-2-thiol. Addition of iodine to the 5-position of o-phenylene thiourea decreases its activity by about 90%.

One of the nitrogens of thiourea may be replaced with oxygen or sulfur with the preservation of some activity. One example is 2-mercaptothiazoline[2] which is slightly more active than thiouracil.

Although it has been found that in several instances when the sulfur of thiourea is incorporated in a ring structure the activity is greatly decreased, this is not the case with 2-aminothiazole. It is of interest that when the C=S bond of thiourea is saturated, as in 2-aminothiazole, activity is not lost.[2]

None of the aminobenzenes has been found to be as active as thiouracil, although 2 have been found[2] to be one-fourth as active, 4,4′diaminodiphenylmethane and bis-(4-dimethylaminophenyl)-methane.

Iodination of aminobenzoic acid did not cause any definite potentiation of antithyroid effect.

With the data available it is not possible to state whether p-aminobenzoic acid and thiouracil have a synergistic effect or whether they have a different site of action. However, they do have an additive effect.

Many of the more potent compounds have been given a clinical trial.[3,13]

Conclusions. Minor structural changes in the 2 main series of goitrogenic compounds, thiocarbonamides and aminobenzenes, may modify the activity of these compounds, sometimes causing a marked potentiation of effect and at other times greatly decreasing or abolishing the effect. The C=S linkage is essential, and usually without substituents attached to the sulfur. In most instances this linkage is adjacent to 2 amino groups, although activity can exist when 1 of the amino groups is replaced by an oxygen or sulfur grouping. Closure of thiourea chains generally causes a potentiation of effect. Additions of short-chain hydrocarbons tend to augment the effect of thiouracil, when attached to the 6-position. Thus far, iodination of the thiourea and aminobenzene derivatives has not augmented the goitrogenic effect. The simultaneous ingestion of thiouracil and p-aminobenzoic acid appeared to result in an additive effect.

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POSTHYPOGLYCEMIC ENCEPHALOPATHY

CASE REPORTS

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THE clinical entity, posthypoglycemic encephalopathy, has been previously reported under headings of synonyms such as fatal hypoglycemia,[40] mental deterioration associated with convulsions and hypoglycemia,[11] cerebral damage from insulin shock,[1] irreversible or hyperglycemic insulin coma,[50] prolonged coma after insulin hypoglycemia,[33] fatal hyperinsulinism with cerebral lesions due to pancreatic adenoma,[23] posthypoglycemic coma or syndrome,[18] and psychiatric complications of hypoglycemia in children.[2]

Even before the introduction of insulin, Joslin[22] recognized that hypoglycemia was a very serious factor in the treatment of diabetes. Wohlwill[49] in 1928 made the first detailed study of brain changes in a diabetic dying from insulin shock, and in the same year Ravid[37] summarized 6 cases of transient hypoglycemic hemiplegia reported in the literature to that date including a case of his own. In the first case of benign tumor of the islands of Langerhans with hypoglycemia proved by antemortem blood studies, reported by McClenahan and Norris,[32] there were evidences of meningeal and cerebral irritation on postmortem examination. Carr,[9] in 1931, reported the case of a diabetic patient with hemiplegia for many weeks after hypoglycemia, and Bast, Schmidt, and Sevringhaus,[6] in 1932, reported a case of hypoglycemia with neurologic symptoms persisting for 5 days after adequate feeding was instituted. At the same time Terplan[43] reported the case of a 16-year old diabetic boy who did not regain consciousness for 3 days after insulin shock, the blood sugar levels being normal for these 3 days. Postmortem examination showed extreme edema of the brain and destruction of ganglion cells. Wilder[47] wrote: "A feature about this type of coma (hypoglycemia) that is very characteristic is its rapid termination when glucose is administered—but recovery is less certain, less rapid and less complete, even when sugar is given, the longer it persists. Irreversible changes may then occur in the brain: degeneration of the ganglion cells, or hemorrhages as described by Terbrüggen." Since that time numerous reports of the same clinical entity have appeared in the literature.

With this introduction, 2 cases personally seen by the author and one other case will be reported:

Case Reports. CASE 1: M. J. W., a 26 year old white male, was first seen at his home on Sept. 8, 1943, at 6 p. m. He had been a known diabetic for 4 years, taking 50 units of protamine zinc insulin daily and not adhering to any special diet. He had had several mild insulin reactions during the preceding two weeks. The day before he was seen he changed his working hours to the midnight to 8 a. m. shift. As his wife worked in the daytime she did not see him at all on Sept. 8th until she returned home at 5.30 p. m. and found the patient lying on the floor, unconscious. The eggs he ordinarily cooked for himself for breakfast were burned to a crisp, and the gas fire beneath them was still burning. It was not known whether he took his insulin that morning.

Examination revealed a young adult white male lying quietly in bed (having been placed there by his wife), perspiring profusely, and unresponsive. Pulse was 88; respirations, 20; blood pressure, 110/86; and temperature, 101.0°. Pupils were dilated and there was normal intraocular pressure to palpation. Teeth were occasionally grinding together. There was no fruity odor to the breath, and the skin was warm and moist. There was carotenemia with a yellowish color to the palms of the hands. Otherwise, a complete

physical examination revealed no abnormality.

The patient was given 30 cc. of 50% glucose intravenously and responded enough to drink about 100 gm. of sugar in solution. In spite of this, his movements became irrational and meaningless and he lapsed back into a stupor. When he was conscious enough to drink he was not able to answer questions or make any meaningful sounds. He was admitted to Baylor University Hospital at 8 p. m. Blood sugar at that time was 460 mg. per 100 cc. and the CO_2 combining power was 40 volumes %. While these results were pending, the patient was given another 125 cc. of 50% glucose intravenously and continuous intravenous glucose was started. The patient became quite unruly and violent, struggling constantly, having to be restrained, and pulled out the intravenous needle after only 300 cc. of 5% glucose had been administered. However, the patient drank 700 cc. orange juice during the night. He continued to be irrational, had occasional mild convulsive seizures, and was frequently mumbling, restless, and trying to get up. Temperature went as high as 102.8° axillary and pulse up to 120/minute.

The 8:30 a. m. blood sugar level on Sept. 9th was 420 mg., and the CO_2 combining power 56 volumes %. Because of the high blood sugar levels the patient was given insulin then for the first time, 25 units of protamine zinc insulin and 15 units of regular insulin, in separate syringes. The patient consumed 1500 cc. orange juice with 60 gm. glucose added and 240 cc. milk orally between then and 3:30 p. m. when his blood sugar level was again determined and said to show no trace of glucose (checked). There was no change in the clinical course during this interval except that the patient screamed irrationally at times. He voided involuntarily. Juices and milk were continued orally and a 9 p. m. blood glucose level was 470 mg. Blood sugar levels checked frequently during the next two days varied from 250 to 460 mg. The patient was given 25 units protamine zinc insulin on each of the next few mornings and he continued to have an adequate carbohydrate intake. He continued to be restless, drowsy, uncooperative, and mentally confused for two more days. When he was finally rational on Sept. 12th, he could not recall anything that had happened for the 24 hours prior to the time he was first seen. Thereafter the patient improved and apparently had an uneventful course after proper regulation of his diabetes.

Case 2. T. A. C., a 55 year old white male, had been a known diabetic since 1938. As he was a bachelor he ate in cafes and remained on no specific diet. He usually took 40 units protamine zinc insulin daily but had decreased his insulin and had been drinking wine prior to being admitted to St. Paul's Hospital in diabetic coma on Feb. 13, 1945. He responded to therapy quite satisfactorily and was instructed on a 3000 calorie, 200 gm. carbohydrate, 100 gm. protein diet, to include a feeding of 30 gm. of carbohydrate before retiring each night. The importance of maintaining the carbohydrate consumption at a constant level was stressed, the patient being kept in the hospital until Feb. 27th in order for him to learn as much as possible about his diabetes, which was well regulated with 60 units protamine zinc insulin and 15 units regular insulin in separate syringes before breakfast daily. His fasting blood sugar level was 90 mg. on the day prior to discharge from the hospital. Because it was believed that the patient possibly would not properly follow his diet, his insulin was decreased to 50 units protamine zinc insulin and 10 units regular insulin on Feb. 26th and 27th. He was discharged on this regimen, having been given diet lists and taught equivalent carbohydrate measures.

In spite of his careful diet instructions it was later learned that he ate only a bowl of soup for his evening meal on Feb. 27th. At 3:00 a.m. on Feb. 28th his landlady heard him making peculiar noises in his room. She entered his room at 7:00 a. m. and reported that he was apparently "normal" at that time. Whether he ate breakfast or took insulin that morning was unknown, but at 10:00 a. m. he was found lying on the floor of his room, unresponsive.

When the patient was seen at 11:00 a. m. he was still unconscious, perspiring excessively. Temperature was 104.0°; pulse, 130; respirations, 28; and blood pressure, 110/60. Pupils were quite irregular in contour, the patient previously having had bilateral cataract extractions. There was excessive salivation, much mucus seemed to be in his throat, crepitant râles and coarse rhonchi were heard throughout both lung fields, and

the patient seemed to have occasional fine tremors over his body.

The patient was given 25 gm. of glucose intravenously but failed to show any response. He was readmitted to St. Paul's Hospital at 12:00 noon on Feb. 28th. As the laboratory technician was not available for blood sugar determination without some delay, 10% glucose solution was started intravenously immediately upon his arrival and was continued until the patient had received 4000 cc. by 10:45 p. m. At 2:30 p. m. the blood sugar level was reported as 164 mg. per 100 cc. The patient was given no insulin and the 8:00 a. m. blood sugar level on March 1st was 53 mg. At 6:00 a. m. on March 1st, nurses recorded that the patient turned himself with ease but was generally unresponsive. As the patient remained unable to swallow, he was given 1000 cc. 10% glucose intravenously at 8:00 a. m., 12:00 noon, and 6:00 p. m. daily, and 1000 cc. 5% glucose intravenously at 12:00 midnight each day thereafter. It was learned that on this program he required and was given only 15 units of protamine zinc insulin daily. Daily 8:00 a. m. blood sugar levels thereafter varied from 74 to 346 mg. Because of the fever and pulmonary edema the patient was given 5 gm. of sodium sulfadiazine intravenously daily. The temperature returned to normal and the lung fields became normal upon examination by March 6th. The patient remained semicomatose on March 1st until 9:00 p. m., at which time he appeared very restless, got out of bed, struggled with the nurses, and had alternating intervals of crying out irrationally and of being quiet. This type of behavior occurred intermittently throughout the night. The patient was incontinent of urine and feces throughout the remainder of his hospital course. He lapsed back into a semicomatose condition on March 2nd and occasionally signified feeling pain when stuck with a needle. On some occasions thereafter he was seen to move his right arm and his right leg but very seldom moved his left arm or left leg. He occasionally mumbled unintelligible sounds and occasionally shook his right arm and fist as if an orator trying to say something. He made occasional grimacing motions and was mentally dull and apathetic throughout his hospital stay. He occasionally coughed but was unable to expectorate, swallow, or speak.

Neurological examination revealed normal triceps, biceps, patellar, and ankle jerk reflexes on the right. Left ankle jerk and patellar reflexes were present but diminished, and the biceps and triceps reflexes were missing on the left. Babinski sign was positive bilaterally. There was no evidence of any cranial nerve involvement. The patient was unable to carry out any command. The heart and vessels were normal except for some tortuosity of the brachial arteries. Abdomen, back, genitalia, rectal examination, and extremities were normal.

On March 5th lumbar puncture revealed clear colorless spinal fluid with pressure of 14 mm. of mercury and no evidence of spinal block. Wassermann and colloidal gold tests were negative and globulin content was normal. White blood cell count was 15 and red blood cell count was 37 per cubic mm. spinal fluid. On March 1st hemoglobin was 11.9 gm. per 100 cc. blood, and white blood cell count 11,000 per c.mm. with a normal differential count. On March 5th, hemoglobin was 10.6 gm. and white blood cell count 7,050 with a normal differential count. Urinalyses were normal except for occasional glycosuria and a terminal pyuria after the patient was catheterized.

The patient remained about the same until March 16th when he became weaker, his temperature began rising, and signs of pneumonia appeared in the lungs. He died on March 18th at 8:20 p. m.

Postmortem examination, as reported by Dr. J. L. Goforth, revealed nothing abnormal externally other than decubitus ulcers in the sacral region. There was marked congestion in the posterior portion of each lung with patchy areas of consolidation noted, grossly suggestive of aspiration pneumonia. Heart examination revealed some dilatation of the right auricle and ventricle, and the left ventricle was fairly well contracted. There were no other gross pulmonary or cardiac abnormalities. No gross nor microscopic abnormality was found in the gastrointestinal tract. The urinary bladder mucosa had an injected appearance, the retention catheter being in place, but there were no other gross abnormalities of the kidneys, prostate, liver, gallbladder, spleen, or lymph nodes. The medullary portion of each adrenal showed what was interpreted as postmortem degeneration. The pancreas was soft and irregularly lumpy with loss of

glandular tissue apparent and no evidence of tumor formation. The brain had a congested appearance but no other gross abnormality. The pituitary body had no apparent changes.

Microscopically there was a moderate degree of myocardial hypertrophy and acute myocardial degeneration; acute spreading bronchopneumonia and a marked degree of pulmonary congestion and edema; congestion and a moderate degree of hyperplasia of the spleen; a marked degree of tubule cell degeneration in the kidneys; diffuse fine scarring and islet degeneration in the pancreas; cortical degenerative changes in the adrenals; and cortical cell degenerative changes in the brain.

A more extensive examination of the brain as reported by Dr. Paul M. Levin revealed moderate hyperemia of the leptomeninges of the cerebral hemispheres. There was an occasional area in the cortex of brownish discoloration, and in other places the tissue seemed more friable than normal. Several radial areas of softening were seen extending from the pial surface through the cortex into the subcortical white matter. The secondary segments of the globus pallidus on both sides showed blurring of the striation and perhaps slight pallor. The region of the third ventricle was normal, as was the remainder of the brain stem and cerebellum. The white matter of the cerebral hemispheres was slightly hyperemic. In places it showed a diffuse brown discoloration. One area of softening was seen in the left occipital lobe; the lateral surface underlay the depth of the calcarine fissures and was curved in a semicircular way so as to conform with the fold of the cortex about this fissure. Sections were cut from both motor areas (from the right including the Rolandic vein in a long block and from the left a deeper block with only pre-central and post-central gyri), wall of third ventricle, globus pallidus, frontal pole, calcarine cortex, and cerebellar cortex. The gross diagnosis was mild hyperemia.

Microscopic examination revealed large areas of cerebral cortex with a regular architecture, composition and vascularity, except for a slight pallor of the ganglion cells. However, in numerous areas of considerable size the cortex had a spongy structure with loss of normal lamination and intense congestion of the blood vessels. The ganglion cells in these lesions were greatly reduced in number; those present were small with pale cytoplasm having an indistinct border. The interstitial cells were greatly increased in number, mostly microglial and phagocytic. Astrocytes were also present in small numbers, often in clumps. These cortical lesions rarely extended deeper than the sixth layer and usually stopped at the fifth; only where they were most severe did the first layer show microglial proliferation. Necrosis was most severe in the third layer where collections of fat-filled phagocytes (glitten cells) were seen; in these areas capillary proliferation was pronounced. The walls of the vessels showed thickening of the walls with endothelial swelling and concentric layers of plump elongated cells. There was also an increase in the number of vessels. The lesions were extensive in the central and occipital cortex. with the least alteration in the frontal poles. The pre-arachnoid was not thickened, but there was a moderate number of macrophages. The globus pallidus showed only a ferruginous deposition in the form of round particles lying in the parenchyma and in walls of blood vessels. The hypothalamus and cerebellar cortex appeared normal. The hypophysis showed a normal structure of the anterior and posterior lobes except for a mild mononuclear infiltration at the margin of the pars nervosa. The microscopic diagnosis was extensive cortical encephalomalacia.

CASE 3: T. J., a 7 year old white male, was never seen by the author but was admitted to Baylor University Hospital at 4:40 p. m. on Sept. 27, 1945, on the service of Dr. W. H. Potts, through whose courtesy the case is being presented. The patient had been a known diabetic since January, 1943, and had last been seen by Dr. Potts in July, 1945. The patient had been on a diet consisting of 60 gm. protein, 120 gm. carbohydrate, and 75 gm. fat daily and had been taking 12 units regular insulin and 10 units protamine zinc insulin daily. He apparently had been getting along satisfactorily on this program except for occasional morning insulin reactions for several weeks prior to his present hospital admission. These were usually satisfactorily relieved by orange juice. On the morning of Sept. 27th when the mother tried to awaken the patient, he had a slight convulsion. He was given orange juice and was later given his usual daily insulin. He ate very little breakfast

and fell to the floor before finishing the meal. As his home was in a town quite some distance from Dallas, the patient was seen by a local doctor, was given another 10 units of regular insulin, and was given more fruit juices and milk. However, the patient vomited what little food he took. Attempts to correct the patient's difficulty by giving glucose intravenously and syrup rectally were apparently unsuccessful. As the patient continued to have convulsions and was comatose it was decided to bring the child to Dallas, the patient being admitted to the hospital upon his arrival.

Upon admission the patient was comatose but the skin was dry. He occasionally groaned and appeared restless. He voided involuntarily. At times he had spastic types of convulsions in the opisthotonus position and had clonic rythmic contractions of both arms in the flexed positions. The eyes became set to the right, and the pupils were dilated. Respirations were shallow and irregular; mucus appeared to have accumulated in the chest and rales and rhonchi were heard over both lung fields; the patient became cyanotic and at intervals had to be given artificial respiration because of apnea. Temperature was 104.0° rectally; pulse, 130; respirations, 30; and blood pressure, 130/80.

Emergency blood sugar level upon admission was 56 mg. per 100 cc. and blood CO_2 combining power was 56 vol. %. The patient was given 70 cc. of 50% glucose and 1000 cc. 5% glucose intravenously. Blood sugar determination at 6:30 p. m. was 421 mg. At 12 midnight the blood sugar level was 222 mg. The patient showed no improvement. He was given 10 units regular insulin at 12:40 a. m. on Sept. 28th. His clinical course continued unchanged. The 8 a. m. blood sugar level on Sept. 28 was 80 mg. and blood CO_2 combining power was 54 vol. %. Lumbar puncture at 8 p. m. on Sept. 27th revealed clear colorless fluid with a pressure of 8 mm. mercury, rising to 20 mm. upon Queckenstedt's test, and then dropping to 10 mm. mercury. The fluid contained 3 white blood cells and 74 red blood cells per c.mm. of fluid. The globulin content was 10 mg. White blood cell count on admission was 7,700 with 1% young, 19% band, and 66% segmented polymorphonuclear cells, and 14% lymphocytes. Hemoglobin was 9.8 gm. and red cell count 3,350,000.

Because of the pulmonary edema and fever, the patient was given penicillin, 20,000 units intramuscularly every 3 hours. He was also placed in an oxygen tent, receiving 7 liters oxygen per minute. Mucus was aspirated from the trachea at intervals. At 9:00 a. m. on Sept. 28th the patient was given 10 units of regular insulin and 500 cc. of 5% glucose intravenously. He received another 500 cc. of 5% glucose intravenously at 2:30 p. m. His condition continued unchanged, there being occasional muscular twitchings and convulsions, with respirations ceasing at irregular intervals. At 6 p. m. on Sept. 28th, respirations ceased permanently.

Postmortem examination was performed by Drs. Howard J. Scott and Charles F. Pelphrey. No gross external abnormality was noted. The lungs were crepitant throughout, pale pink in color anteriorly and slightly dark red posteriorly. Multiple sections revealed a homogeneous appearance with pink watery fluid exuding from the cut surfaces of both lungs. Occasional areas showed slight mottling, however, not interpreted grossly as bronchopneumonia. The heart was grossly normal. There was some passive hyperemia of the spleen and fatty degeneration of the liver. Passive hyperemia and cloudy swelling were found in the kidneys. Pancreas, adrenals, and other abdominal viscera were normal in appearance. Examination of the brain showed cerebral edema and scattered numerous small dark red spots which did not rub off easily, suggestive of petechial hemorrhages.

Microscopically there were multiple recent capillary hemorrhages in the heart; hypostatic edema, marked hyperemia, and early hypostatic pneumonia with recent small hemorrhages in the same area of lungs; hyperemia and acute parenchymatous degeneration of the kidneys; no evidence of substantial glycogen storage in the liver; hyperplasia of the malpighian corpuscles of the spleen; islets of Langerhans small with cells having a scanty amount of cytoplasm; hyperemia and hyperplasia of the medulla of the adrenals; and marked hyperemia, lymphocytic hyperplasia, and acute lymphadenitis of the mesenteric lymph nodes.

A less extensive study of the brain was made than that reported in Case 2. However, later study of the sections available, as reported by Dr. Paul M. Levin, revealed moderate to marked hyperemia and slight edema in some areas of the cortex and prob-

able diffuse loss of ganglion cells from the second and third layers of cerebral cortex.

Comment. Dameshek, *et al.*,[10] have shown that the difference in dextrose content between blood drawn from the carotid artery and the internal jugular vein was greater than that between the brachial artery and the basilic vein, and Himwich[20] has shown that oxygen utilization and the metabolic rate of the brain are decreased during severe hypoglycemia. Gerard[16] reported that the respiration of the brain, that is, oxygen consumption per unit mass, is about thirty times as intense as that of muscle or nerve. It is generally agreed that dextrose is essential in normal utilization of oxygen by brain tissue and that acute anoxia or hypoglycemia affects first the cerebral cortex, next the upper portion of the brain stem, and finally the medulla.[15,16,20,20a,21,29,31,44]

Grayzel[19] reported that rabbits in which hypoglycemia was experimentally produced showed definite central nervous system lesions and that zones of necrobiosis were more noticeable in the pyramidal cells of the third and fifth cortical layers.

Other animal experimentation has shown hypoglycemia to produce liquefaction, vacuolization and homogenization of ganglion cells, marked shrinkage of cytoplasm and nuclei, diminution in number of neurons in various cortical areas, and small microscopic hemorrhages.[7,41-45] Sherrill and Mac Kay[42] reported a variable degree of hemorrhagic edema of the lungs in experimental animals with hypoglycemia, probably similar to the pulmonary edema seen in Case 2 when the patient was first seen in hypoglycemia and similar to the pulmonary edema seen in Case 3. Baker[3,4,5,27] at first believed that changes found in ganglion cells on postmortem examination of 3 patients dying after hypoglycemia were due to postmortem degeneration but in later papers concluded that these were antemortem changes. He subsequently reported extensive brain damage, congestion, hemorrhages, patchy demyelinization, cyst formation, encephalomalacia with necrotic and active proliferation, various degrees of cellular degeneration, and gliosis following hypoglycemia. Numerous other similar reports have now appeared.[23,25,26,35,38,40,45] "It is indeed difficult to distinguish less severe nerve-cell changes from postmortem alterations."[26]

Schereschewsky and others have reported necrosis and hemorrhage into the adrenal glands after hypoglycemia.[25,36,41] Perhaps the adrenal gland changes reported in Case 2 were antemortem changes rather than postmortem degeneration. If so, this could account for the patient's decreased insulin requirement after his hypoglycemia although he continued to receive 350 gm. carbohydrate daily.

Clinically, cases of posthypoglycemic encephalopathy have been reported in every age group, as frequently in children as in adults. Rascoff *et al.*[36] reported a case of hypoglycemia of the newborn with hypertrophy and hyperplasia of the islands of Langerhans, dying 13 hours after birth from a latent diabetic mother. Although posthypoglycemic encephalopathy, to the author's knowledge, has not definitely been reported following hypoglycemia of the newborn, it remains a definite possibility to be considered in determining the etiology of mental deficiency states of childhood. Darrow[11] had 2 cases of convulsions and mental deficiency in children following hypoglycemia. A case of transitory hemiplegia associated with hypoglycemia in a diabetic child with congenital heart disease was reported by Fischer.[14] Allan and Crommelin[1] reported the case of a 6 year old diabetic with an insulin reaction in which the patient was stuporous for 3 days, followed by hemiplegia, aphasia, and epileptic seizures for 15 months. In Wilder's case[48] an 8-year old child had idiocy secondary to a degenerative lesion of the brain from overdosage of insulin. Unconsciousness persisted for 6 weeks, and the mental condition never returned to normal. Anderson[2] had a 7-year old diabetic patient with a severe behavior disorder following hypoglycemia and a 13-year old diabetic with an insulin reaction followed by 4 days of coma and then gross dementia

which gradually but only partially improved during the next 15 months. Fenz and Kogerer[13] reported the case of a 16-year old boy in which a severe insulin reaction was followed for a fortnight by a syndrome characterized by negativism, catalepsy, grimacing, and motor restlessness, with subsequent improvement but persistence of apathy and dulness after 4 months. Terplan's case[43] has already been mentioned.

Numerous similar cases have been reported in adults, not only in diabetics,[8,14,46] but also following the Sakel form of shock therapy for schizophrenia,[12,17,34,39] and in cases with pancreatic adenoma.[6,23,30] The case reported by Root and Styron[38] in which the patient remained in a comatose state for 23 days and finally died with terminal bronchopneumonia and sepsis is similar to Case 2. Another similar case with coma for 17 days terminating in death is reported by Lawrence *et al.*[26] Cases with Korsakoff's syndrome persisting for several months after hypoglycemic shock have been seen.[24,33] Also found are cases in which a combination of hypoglycemia and arteriosclerosis seemed to cause the cerebral damage.[28] Murphy and Purtell[35] reviewed 26 cases of hypoglycemia with cerebral damage and concluded that the most common aftermath was profound mental and personality damage, extensive damage leaving the patient with the intellectual level of idiocy, less damage resulting in mental retardation, apathy, and dulness, at times progressing to true psychoses. Parkinsonism, aphasias, hemiplegias, and objective neurologic changes have persisted for months.

Goldman concluded: "Coma persisting after cessation of hypoglycemia is an entity in itself and related only in a secondary sense to the hypoglycemia."[18] Thus, in Case 1 a state of mental confusion and change in personality, apparent clinical persistence of hypoglycemia, remained for 3 days after he was first seen and for 48 hours with high blood sugar levels after the 3:30 p. m. blood was reported as showing no trace of glucose the day after admission to the hospital. This drop in blood sugar level under these circumstances was undoubtedly the result of loss of liver glucose by glycogenolysis during the severe insulin reaction on the preceding day and indicates the high degree of sensitivity of an individual to insulin after a severe hypoglycemic episode and the necessity of continuing high carbohydrate intake in spite of high blood sugar levels in such cases. The clinical picture was not due to persistent hypoglycemia but to transient cerebral damage caused by the preceding hypoglycemia. In Case 2, apparent idiocy, aphasia, and neurologic changes remained for 18 days after the blood sugar returned to normal or high levels. Case 3 died after having remained comatose with occasional muscular twitchings, convulsions, and irregular respirations for 24 hours after termination of hypoglycemia. The term, posthypoglycemic encephalopathy, although to the author's knowledge not previously used in the literature, seems to be the preferable descriptive term for this clinical entity.

Conclusions. Numerous cases of personality, mental, and neurologic changes following hypoglycemia and persisting after the blood sugar levels have become normal have been previously reported.

The physiological and pathological changes responsible for the clinical behavior are briefly discussed.

Three cases belonging in this clinical group are reported, one with mental confusion and personality changes for 48 hours after cessation of hypoglycemia; one with marked mental impairment and persistent neurologic finding for 18 days, terminating in death; and a third terminating fatally with coma and convulsions for 24 hours after the hypoglycemia was satisfactorily controlled. Postmortem findings in the last 2 cases are reported.

It is recommended that the term posthypoglycemic encephalopathy be used for this very definite clinical entity.

Members of the medical profession should be aware of this very serious complication of hypoglycemia and combat any prolonged low blood sugar level vigorously.

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PLASMA PROTEINS

II. Alteration in Alloxan Diabetic Rabbits Especially in Relation to Ocular Damage

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In previous studies changes in the plasma protein distribution in clinical diabetes mellitus as measured by the Tiselius electrophoresis method were reported.[5] Reduction in total albumin and elevation of the β-globulin fraction were demonstrated in uncontrolled diabetes. With adequate treatment, a return to normal occurred. It was also suggested that cases complicated by diabetic retinitis showed a still greater deviation from normal and distinctly less improvement with diabetic control.

Alloxan administered in appropriate amounts results in the production of permanent islet cell damage in rabbits, rats and dogs.[2,3,4] In this study on alloxan diabetic rabbits we wished, first, to determine what modification, if any, occurred in plasma protein fractions, and, second, to note the progress of these changes with different combinations of insulin, diet and vitamins. During the course of the studies the eyes were examined for lens and retinal changes.

Animals. In early studies 7 albino rabbits, 4 Belgian hares, and 5 others of a mixed strain were used. Later studies were made on Belgian hares, because of the more even distribution of retinal pigment. In the albino visualization of fundus vessels is more difficult.

Twenty-four rabbits were used. Alloxan, 100 mg. per kg. body weight, was administered intravenously to 20; 2 were kept as normal controls. Two rabbits received repeated small injections. Three animals were bled repeatedly prior to administering alloxan in order to lower plasma albumin levels and to witness the effects of this upon susceptibility to later retinal hemorrhage. If evidence of hypoglycemia was manifested at any time during the 24 hours after injection, 50% dextrose solution was given intravenously. The 2 normal control rabbits were treated identically insofar as bleeding, diet and examinations were concerned.

Methods. Blood glucose was determined by the Benedict blood sugar method before and after development of diabetes. Urinary glucose was determined qualitatively or quantitatively by the Benedict method. To determine the occurrence of chemical nephritis, the urine was examined for albumin and red cells.

The D/N ratio was determined on aliquot portions of a 24 hour urine specimen collected from the 48th to the 72nd hour of a 72 hour fast. Nitrogen was determined by the Pregl modification of the micro-Kjeldahl method.

The Tiselius electrophoretic technique was used for plasma protein fractionation.[4] Blood was drawn from the femoral artery. Potassium oxalate was used as anticoagulant.

Diet. Diet 1 consists of oats *ad lib.* and approximately 4.5 ounces of carrots and 4 ounces of greens daily (celery greens, cabbage, lettuce or green hay). Approximately 2 ounces of oats daily were eaten by most of the rabbits.

Diet 2 consisted of Purina rabbit pellets *ad lib.* and either celery or lettuce greens. The average rabbit consumed 3 to 4 ounces of pellets a day, 4.5 ounces of carrots, and 4 ounces of greens.

Eye Examination. The eyes were examined after maximum dilatation of the pupils with 1% atropine sulfate solution. The day before the examination 2 drops were administered about 5 P.M. The next morning drops were given about 9 A.M. in preparation for a noon examination. If dilatation was not complete, 2 drops more were given 2 hours later. If more rapid dilatation was necessary 2 or 3 doses were administered at ½ hour intervals. When capillary hemorrhages were observed, the examinations were always viewed by a second ophthalmologist. Rabbits with and without hemorrhages and with and without diabetes were presented at random to the examiner, who was thus entirely unbiased.

Results. As previously reported, increased blood sugar and glycosuria were observed within 48 hours after injection of alloxan (100 mg. per kg. or more) into normal rabbits. After the initial injection, 18 rabbits developed permanent hyperglycemia. In 1 it appeared only after a second injection. One rabbit failed to show more than temporary elevation in blood sugar even after the second administration; 1 failed to become hyperglycemic after 2 injections. In 2 rabbits diabetes was produced by giving repeated smaller amounts of alloxan. One received 6 injections of 20 mg. per kg., whereas the other received 5 injections of 40 mg. per kg. at intervals of 3 days.

Acetonuria was observed in 6 animals from the 4th to 12th day following development of diabetes. In 5 cases it was controlled by protamine-zinc insulin. On withholding therapy, ketosis occurred in only 1 case. This animal required 5 units of protamine-zinc insulin daily for the 1st month to combat signs of diabetic acidosis. From the 2nd to 5th month inclusive, insulin in similar dosage 3 times a week prevented ketonuria. Therapy was then stopped, and the animal appeared in good condition until food was withheld for 72 hours in order to determine the D/N ratio. Severe acidosis resulted and death followed despite treatment with large amounts of insulin and intravenous fluids. The animal had moderately severe diabetes as judged by the D/N ratio, 2. Some animals, however, had even higher ratios, but had less tendency to excessive ketone body production. All of the animals in this group were in about the same nutritional condition and received the same diet.

As was previously observed in untreated diabetic patients, rabbits with severe diabetes and acidosis usually showed a decrease in albumin, and a pronounced increase in the β-globulin fraction (Tables 2 and 3). When ketosis ceased and the animal's condition improved, either after treatment with insulin or after some spontaneous adjustment, the β-globulin returned to normal. In insulin-treated animals the albumin level frequently increased.

Rabbits with mild diabetes (Table 1, R-8) usually showed a decrease in total plasma protein and albumin but no significant modification in β-globulin. When insulin therapy reduced glycosuria to a trace to 1+, the total protein and albumin levels tended to return to normal.

Animals with moderately severe diabetes (Table 1) but without acidosis showed plasma protein shifts of a degree comparable to those observed in uncomplicated diabetes in man.

During the course of diabetes in the rabbit many animals showed eye changes. Modifications in the plasma protein picture were not necessary accompaniments of developing lens opacities. On the other hand, a low plasma albumin and an elevated β-globulin were nearly always found at the time that fine capillary bleeding in the retina was observed. The interval before appearance of eye changes varied greatly from animal to animal (Table 4) and seemed to have little correlation with the severity of the diabetes, loss of weight, or diet. One rabbit (D/N 0.7) showed lens vacuoles 6 weeks after, and fundus changes 4½ months after islet cell damage was produced. In another animal with mild diabetes (D/N 0.5) 3 months elapsed before lens changes and 3½ months before

capillary bleeding were noted. However, 1 animal (D/N 0.75) showed capillary hemorrhages 1 month and lens cloudiness 2 months after onset of diabetes.

Of the 21 diabetic rabbits, 18 showed progressive lens changes, apparently quite similar to those described by Bailey and Bailey.[1] Six rabbits were given large amounts of vitamins in addition to protamine-zinc insulin in an attempt to prevent or delay development of lens changes (Table 1). None of the group failed to show lens opacities. Because of the wide variation in the time required for these changes to occur in the untreated diabetic rabbit as well as in the treated animal, a much larger series will have to be studied before any conclusions as to the effect of vitamin therapy can be drawn.

In 8, retinal changes, which in some instances persisted for 6 weeks, consisted of very fine, minute capillary bleeding along the larger vessels. It was possible to follow pigment deposition at the site of bleeding as the hemorrhages were absorbed. No exudates have been observed either clinically or on microscopic examination of the retinæ. Apart from minor retinal changes, none have been observed to compare with the severe retinal degeneration in diabetes mellitus in man.

Microscopic examination of the eyes of 4 of the rabbits was made. In Rabbits R-3, R-7 and R-17 (see illustration), the

TABLE 1.—PROTOCOL OF DIABETIC RABBIT 2

Brown and white. Alloxan injections 4/3/44 and 4/27/44.

	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Mar. 1945
Fasting blood glucose, mg./100 ml.	75	250	350	....	....	....	....	350	
Urine:									
Glucose, gm./24 hr.	0	22	12 21	....	Trace	0 to trace			
Acetone	0	4+	0 2+	....	0	0			
Protamine-zinc insulin, U/24 hr.	..	..	....	6/26	....	8/26 3			
Vitamins*	..	..	....	9/14					
			5/9	6/21	7/12	8/10	9/29	..	3/2
Lens changes OD	Clear	..	Diffuse central opacities	Progressing	Early post-central	Central remain same	Much more definite central and periphery	..	Definite, especially central; extreme clouding of lens
OS	Clear	..	OS OD	Progressing	....	Central remain same	Much more definite central and periphery		
Retina	Normal	..	....	....	..	Occasional vacuoles in periphery	Not clearly seen		
Total plasma protein	3/30	4/20	5/16	....	....	....	....	10/2	
Gm./100 ml.	5.18	4.99	4.84	....	....	....	....	5.87	5.86
Albumin:									
Gm./100 ml.	3.20	3.19	3.05	....	....	....	....	3.41	3.62
%	61.80	63.80	63.10	....	....	....	....	59.10	61.80
α-Globulin:									
Gm./100 ml.	0.17	0.27	0.20	....	....	....	....	0.19	0.15
%	3.20	5.40	4.10	....	....	....	....	3.30	2.70
β-Globulin:									
Gm./100 ml.	0.92	0.74	0.72	....	....	....	....	0.89	0.70
%	17.80	14.90	14.90	....	....	....	....	15.40	11.90
γ-Globulin:									
Gm./100 ml.	0.52	0.40	0.54	....	....	....	....	0.77	0.78
%	10.00	8.00	11.10	....	....	....	....	13.40	13.20
Fibrinogen:									
Gm./100 ml.	0.37	0.39	0.33	....	....	....	....	0.51	0.61
%	7.20	7.80	6.80	....	....	....	....	8.80	10.40

* Daily dose.
Vitamin A, 10,000 U.
Thiamin chloride, 3 mg.
Riboflavin, 6 mg.
Pyridoxine hydrochloride, 1.5 mg.
Nicotinamide, 30 mg.
Calcium pantothenate, 10 mg.
Desiccated fraction of liver-stomach concentrate with yeast extract 0.6 gm.
Ascorbic acid, 50 mg.
Liver extract (Lilly), 1 cc.

TABLE 2.—PROTOCOL OF RABBIT 3

♀ Albino. Alloxan injection 4/3/45 and 4/13/45.

	March	April	May	June	July	August	September	October	November	January
Fasting blood glucose, mg./100 ml.	72	..	5/8 320	....	100	....	9/6 120	10/10* 225	....	315
Urine glucose, gm./24 hr.	0	..	31	....	3	....	....	..	....	21
Acetone	..	..	4+							
Protamine-zinc insulin, U/24 hr.	..	..	0	6/26	3	8/24	....	4	11/17	0
Lens changes:										
OD	..	..	Clear	Clear	Clear	Fine postcentral lens changes	More marked postcentral and early periphery lens changes	..	More marked	Lens changes extensive
OS	..	..	Clear	Clouding	Central lens changes three dots	Same	9/27 same	..	More marked	Lens changes more extensive than OD
Retinæ	..	..	5/9 two fine capillary hemorrhages in fundus OD 5/27 hemorrhage being absorbed	6/23 new hemorrhage OD 6/29 no new hemorrhage in either eye	....	8/7 no new hemorrhage	9/27 fine hemorrhage anterior OS	..	11/17 not clearly visualized	1/16 not visible
Total plasma protein:	Serum	5/8	5/23							
Gm./100 ml.	5.84	4.47	4.97	4.86	....	5.22	5.42	..	5.79	5.47
Albumin:										
Gm./100 ml.	4.19	2.50	2.80	2.13	....	3.20	3.46	..	3.44	3.25
%	71.80	55.90	56.20	43.90	....	61.20	60.30	..	59.40	59.40
α-Globulin:										
Gm./100 ml.	0.33	0.47	0.40	0.42	....	0.36	0.40	..	0.48	0.29
%	5.70	10.60	8.10	8.60	....	6.90	7.40	..	8.30	5.30
β-Globulin:										
Gm./100 ml.	0.60	0.86	0.89	0.78	....	0.49	0.61	..	0.68	0.66
%	10.20	19.30	18.00	16.00	....	9.40	11.20	..	11.80	12.10
γ-Globulin:										
Gm./100 ml.	0.72	0.37	0.52	0.85	....	0.53	0.60	..	0.72	0.79
%	12.30	8.10	10.40	17.40	....	10.20	11.00	..	12.40	14.50
Fibrinogen:										
Gm./100 ml.	..	0.27	0.36	0.68	....	0.64	0.55	..	0.47	0.48
%	..	6.10	7.30	14.10	....	12.30	10.10	..	8.10	8.70

Conclusions: 1. Before insulin: total fell; albumin fell appreciably; 2 hemorrhages occurred; some rise in all globulin fractions
2. After insulin: total rose and approached normal; albumin tended to rise; globulin fractions tended to fall, none reaching normal, however.

* Acute upper respiratory tract infection.

abnormal changes were all of similar nature, but varied in degree. In the sections of the eyeballs, in the posterior part of the retina, on both sides of the entrance of the optic nerve, for a considerable distance, there were some small circular, irregular or elliptical disk-like foci of amorphous granular, deeply eosinophilic material, situated beneath the inner limiting membrane and partly occupying or were present just beneath the inner limiting membrane. No obvious degenerative or inflammatory disease of the arterioles was detectable, so that the disks of eosinophilic material, if they did represent old hemorrhage, must have been of capillary origin. The fact that the blood elements were unrecognizable would indicate that these small foci of hemorrhage were not recent. The only other pathologic change

TABLE 3.—PROTOCOL OF RABBIT 24

B. H. Alloxan 100 mg./kg. injection 8/25/44.

	August	September	October	November	January
Fasting blood glucose, mg./100 ml.	78	450	....	....	290
Urine:					
Glucose, gm./24 hr.	0	36	....	....	31
Acetone	0	2+	....	....	0
D/N ratio	...	....	....	....	0.91
Lens changes:					
OD	Normal	Lens spot at 6 o'clock	Early peripheral	More marked peripheral diffuse central	Diffuse so dense retina could not be seen
OS	Normal	....	Early peripheral	More marked peripheral diffuse central	
Retinæ	Normal	6 fine capillary hemorrhagic areas along posterior vessels OD OS normal	Hemorrhage in both eyes numerous	Hemorrhage still present	
Total plasma protein:	Serum	9/12/44			
Gm./100 ml.	4.74	4.59			
Albumin:					
Gm./100 ml.	3.38	2.26			
%	71.20	49.20			
α-Globulin:					
Gm./100 ml.	0.20	6.30			
%	4.20	6.50			
-Globulin:					
Gm./100 ml.	0.56	1.24			
%	11.90	27.00			
γ-Globulin:					
Gm./100 ml.	0.60	0.40			
%	12.70	8.70			
Fibrinogen:					
Gm./100 ml.	...	0.39			
%	...	8.60			

Diet: oats, carrots and greens.

NOTE.—Severe diabetes in an untreated female rabbit showing very marked shifts in total albumin and β-globulin fractions. Changes may have been intensified and hastened by repeated plasmaphoresis prior to administration of alloxan.

Bled 20 ml. 8/22, 23 and 24. Cells returned.

compressing the ganglion layer. Within these foci there were some pale, minute golden brown pigment granules of variable size. Similar pigment granules were also present in the ganglion layer in this region. The disk-like eosinophilic foci suggested agglomerated, disintegrated red blood corpuscles but definite corpuscles could not be identified. In the ganglion layer, especially in the region of the eosinophilic disks, there were a moderate number of dilated capillaries filled with red blood corpuscles and some of these capillaries in these eyeballs was lenticular cataract, which was most marked in the eyes of R-7 and only beginning in R-3 and R-17.

R-31 did not show the changes in the retina described above. The only change observed was vacuolization in the optic nerve. The nature and significance of this was not determined.

It may be noted that fundus examination of R-3, R-7 and R-17 had shown considerably more extensive capillary hemorrhages than were observed in R-31.

Where plasma proteins were experimen-

TABLE 4.—RELATION OF SEVERITY AND DURATION OF ALLOXAN DIABETES TO DEVELOPMENT OF EYE CHANGES IN RABBITS

Rabbit and breed	Severity of diabetes				Duration of diabetes at onset	
	Blood glucose (mg./100 ml.)	Urine				
		Glucose (gm./24 hr.)	Acetone	D/N	Lens changes (wks.)	Capillary hemorrhages (wks.)
2M	340	22	2+ for 2 wks. after initial diabetes; none later	..	3	
3A	340	31	4+ for 4 wks.; 0 to trace afterward; 21st wk. 2+	..	8	5
5A	350	17	4+ to trace for 3 wks.	..	5	
7A	235	17	0	0.79	5 Occasional vacuoles	17
8A	200	12	0	0.50	12	14
13BH	325	20	1+ for 1 wk.	..	2	
14B	255	13	0	..	4	
15G	190	Trace	0	..	16	
17BH	280	20	0	..	3	14
18A	254	22	0	0.70	8	10
23A	400	24	0	1.00	2	
24BH*	450	36	1 to 2+ 2nd and 3rd wk.	0.91	3	1
25BH*	300	23	Trace 1st wk.	1.75	3	1
27BH*	..	23	0	..	..	Aneurysmal dilatation of 1 venule
28BH†	..	31	4+ 1st wk.	2.00	6 Corneal scar and haziness 15 Lens opacities both eyes	
31BH†	..	10	0	0.75	6	3
32BH†	..	35	0	2.35	5	
35BH	..	32	0	3.58	5	
36BH	..	28	4+ 1st week	..	3	
40BH	..	25	.....	0.75	3 1 large vacuole	
1BH normal control	..	0	0	0	Normal for 26 wks. observed	
33BH† normal control	..	0	0	0	Normal for 12 wks. observed	
29BH‡	90	0	0	0	Normal for 24 wks. observed	

* Repeatedly bled before alloxan. Diet: oats unless marked † indicating Purina diet.
‡ Apparently resistant to alloxan. No diabetes, after 2 intravenous injections of 100 mg. per kg.
A, albino; B, brown; M, mixed; GR, gray; BH, Belgian hare.

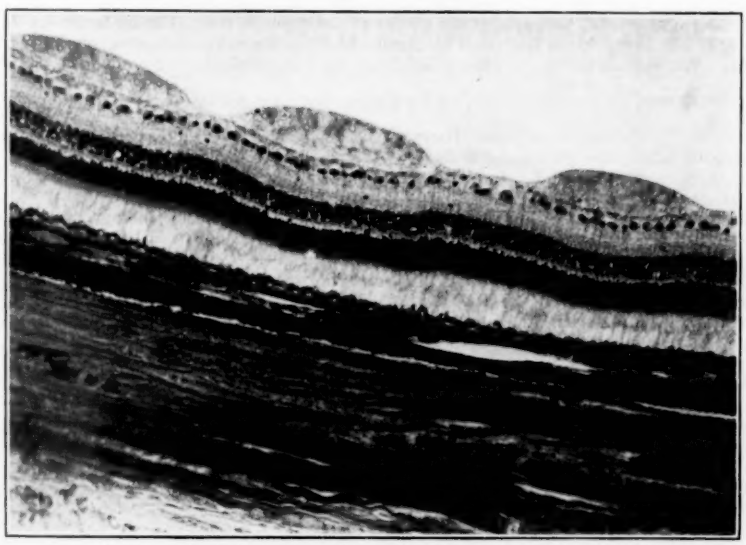

FIG. 1.—Typical changes observed in the right retina of R-17

tally depleted by plasmapheresis before the production of diabetes, retinal changes developed more rapidly. The fundi were examined after plasmapheresis and prior to alloxan administration to be sure that depletion of plasma proteins alone was not responsible for capillary bleeding.

The toxic effect of alloxan was probably not responsible for the capillary bleeding, for in some cases it was first observed 3 to 4 months after administration of the drug.

In 2 normal rabbits and the 1 which failed to become diabetic after 2 injections of alloxan, the eyes were normal on all examinations. These rabbits received the same diets as the diabetic animals. They were bled and the retinæ examined at the same intervals.

Summary. Plasma protein fractionations (Tiselius technique) were made on 24 rabbits before and after the development of alloxan diabetes. In severe diabetes with acidosis there was a marked increase in the β-globulin and a decrease in the albumin level. With disappearance of acidosis, either following insulin treatment or spontaneous adjustment, the β-globulin level became normal. In some cases with insulin therapy, the albumin approached the normal value.

In mild diabetes, without acidosis, there was frequently a decrease in the total plasma protein, but little change in the relative percentage of the various fractions.

Twenty-one of the rabbits showed progressive lens changes and 8 showed minute capillary bleeding in the retina. The plasma albumin was usually markedly decreased and the β-globulin increased at the time that hemorrhages were observed.

The rabbit which failed to develop diabetes following injection of alloxan also failed to show any plasma protein changes or eye abnormality.

Conclusions. Plasma protein changes which occur in the rabbit with alloxan diabetes (*i. e.*, a marked increase in the β-globulin and decrease in albumin levels) are similar to those which are observed in human diabetes. The development of ocular complications in rabbits demonstrates a further similarity between diabetes mellitus in man and diabetes produced by alloxan.

We wish to express our sincere appreciation to: Dr. F. B. Peck of the Lilly Research Laboratories for generous supplies of vitamins and insulin; Dr. J. Murray Scott of Ayerst McKenna & Harrison, and Dr. Merton C. Lockhart of the Lederle Laboratories, Inc., for vitamins. Mr. James Clark gave valuable technical assistance throughout the course of the experiments. We are indebted to Dr. A. D. Ruedemann, Dr. E. Perry McCullagh and Dr. Irvine H. Page for aid and suggestions during the course of this work. We wish to thank Dr. Harry Goldblatt for the histologic examination of the rabbit eyes.

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A ROUTINE METHOD FOR THE RAPID DETERMINATION OF SUSCEPTIBILITY TO PENICILLIN AND OTHER ANTIBIOTICS

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It is reasonable to assume that a variety of antibiotic agents will be available to the physician in the near future. Already penicillin and tyrothricin are available for the treatment of many infections caused by gram positive organisms. Although still in the experimental stage, streptomycin shows promise in combating diseases due to gram negative bacilli. No doubt other antibiotics will be developed with more or less specific activity for certain types of bacteria.

When several antibiotic agents are available, the clinician will be faced with the problem of selecting the correct one. Among the factors which will influence his decision, the most important is the relative susceptibility of the infecting organism. For example, *H. influenzæ* is moderately resistant to penicillin[7] but highly susceptible to streptomycin; pneumococci are penicillin susceptible but many strains are quite resistant to streptomycin. Neter[13] studied the relative susceptibility of staphylococci to penicillin, tyrothricin and streptothricin and found a variety of susceptibility patterns. Resistance to 1 of the antibiotic agents does not appear to be related to resistance to the other 2. Strains of the same species not only display variation in relative susceptibility to 2 different antibiotics but they also show marked differences in their susceptibility to a single antibiotic.[5] For example, Bondi and Dietz[1] found 13.9% of 116 freshly isolated staphylococci to be resistant to penicillin. Buggs and his associates[3] have likewise shown that there is great variation in the susceptibility of strains of the same species to streptomycin.

In view of the increasing number of available antibiotics, it is apparent that adequate bacteriologic study of infectious diseases should include routine tests for susceptibility. Several methods[10,14] are available for determining susceptibility to penicillin. These procedures, however, require first the isolation of the organism in pure culture and on the following day the determination of susceptibility. Although the results are reasonably accurate, a delay of 2 days from receipt of the specimen is inescapable. Furthermore, in the instance of a mixed infection separate determinations must be carried out for each organism. A simple and more rapid test would be desirable even at the sacrifice of some degree of accuracy. Such a test is described in this paper.

The procedure to be reported is a modification of that described by Vincent and Vincent[15] for the assay of penicillin. By placing filter paper disks saturated with antibiotic solution on the surface of a blood agar plate immediately after inoculation with a clinical specimen, it is possible to test susceptibility at the same time the primary culture is made. Following overnight incubation susceptibility of the organisms is determined by measurement of zones of growth inhibition surrounding the disks. Thus, an evaluation of susceptibility can be obtained within 24 hours, regardless of whether the culture contains 1 or several types of bacteria. Furthermore, as many as 3 different anti-

biotics may be compared simultaneously, although to date the authors' experience is limited to the use of penicillin and streptomycin.

Method. In this laboratory each specimen is streaked in an identical manner on 2 blood agar plates, 1 each for aërobic and anaërobic incubation. The medium is a veal infusion agar prepared according to the method of Wright[16] to which is added 2% tryptose and 10% defibrinated horse blood. The swab or loopful of fluid is drawn once across one side of the agar surface. This primary inoculum is restreaked with a sterile inoculating loop and is spread with parallel streak-

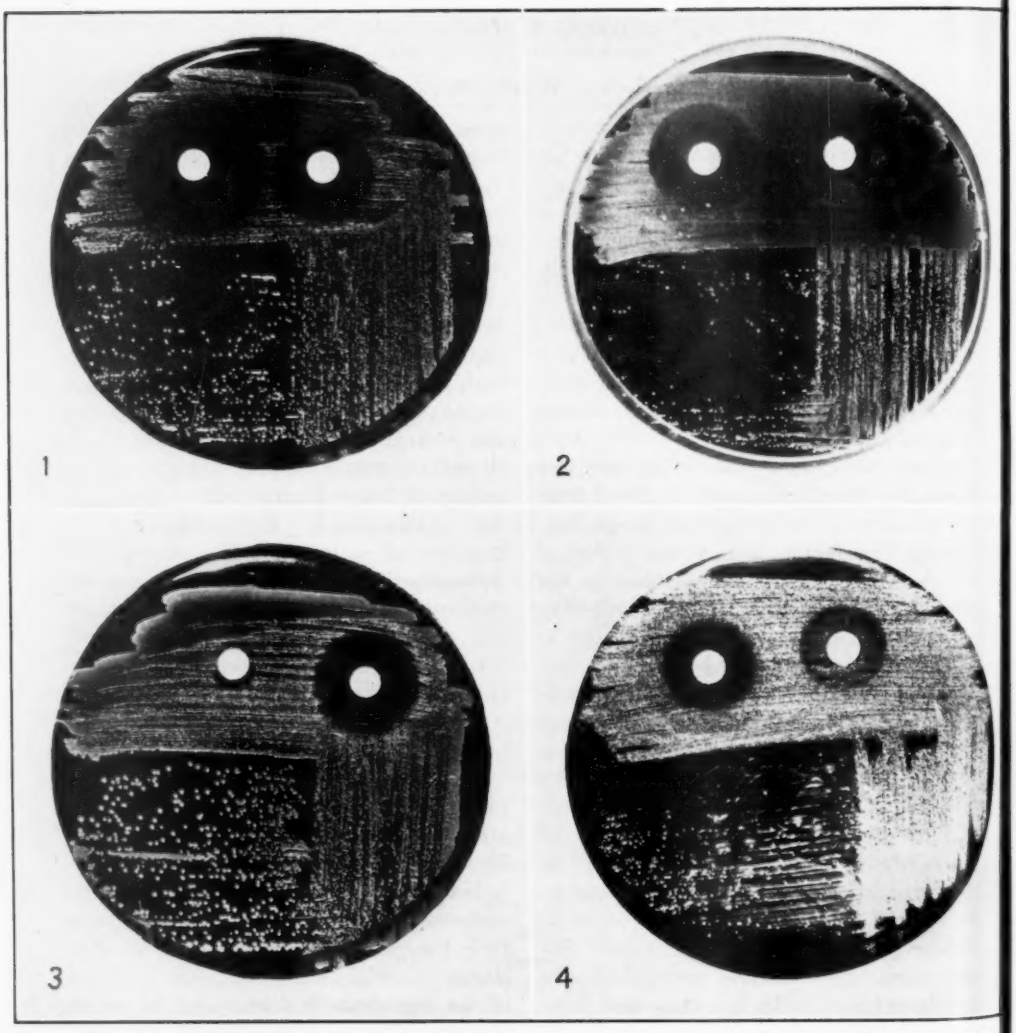

FIG. 1.—Disk susceptibility tests: veal infusion agar with 2% tryptose containing 10% defibrinated horse blood. In each figure disk on left side of plate is saturated with penicillin (15 units/ml.), disk on right saturated with streptomycin (500 μg./ml.). *1*, *Staph. aureus;* very susceptible (26 mm.) to penicillin; very susceptible (19 mm.) to streptomycin. *2*, Hemolytic streptococcus; very susceptible (21 mm.) to penicillin; resistant (9 mm.) to streptomycin. *3*, *Staph. aureus;* resistant to penicillin; very susceptible (19 mm.) to streptomycin. *4*, Enterococcus; moderately susceptible (17 mm.) to penicillin (small inner zone); resistant to streptomycin. Hemolytic streptococcus; very susceptible (27 mm.) to penicillin (large outer zone); very susceptible (16 mm.) to streptomycin.

ing over one-third of the surface of the plate. After flaming the loop the plate is successively turned at right angles and streaked in the same manner, care being taken to touch the previous inoculum with each streak until the entire surface has been covered.

following preliminary trials with various strengths of each.

Following overnight incubation, both plates are examined for colony identification and for evaluation of the susceptibility of the predominant or pathogenic types present. Susceptibility is evidenced by the

TABLE 1.—RELATIVE SUSCEPTIBILITY BY THE DISK METHOD IN TERMS OF UNITS

Agent	Zone of inhibition; mm.	Susceptibility range	Relative susceptibility
Penicillin	>20	<0.1 units/ml.	Very
	10–20	0.1–0.4 units/ml.	Moderate
	<10	>0.4 units/ml.	Resistant
Streptomycin	>15	<4 μg./ml.	Very
	10–15	4–15 μg./ml.	Moderate
	<10	>15 μg./ml.	Resistant

TABLE 2.—SUSCEPTIBILITY OF BACTERIA* BY DISK METHOD

Organism	No. strains	Penicillin			Streptomycin		
		Very	Mod.	Resist.	Very	Mod.	Resist.
β-Hemolytic streptococci	17	15	2	0	1	2	14
Viridans streptococci	10	8	1	1	0	2	8
Non-hemolytic streptococci	9	5	0	4	1	2	6
Enterococci	4	2	1	1	0	0	4
Staphylococci	8	6	0	2	7	1	0
Pneumococci	10	8	2	0	0	1	9
E. coli	11	0	0	11	8	3	0
A. aerogenes	2	0	0	2	2	0	0
Klebsiella	1	0	0	1	1	0	0
Proteus	2	0	0	2	2	0	0
Ps. pyocyanea	6	0	0	6	0	2	4
H. influenzæ	16	0	1	15	11	5	0

* The significant organisms encountered in 54 selected clinical specimens.

Disks of Whatman No. 2 filter paper, 6.5 mm. in diameter, are cut with a cork borer or paper-punch; these are sterilized in a petri dish in the hot-air oven. By means of alcohol-flamed, fine-pointed forceps 1 of the disks is dipped into a solution of commercial penicillin carrying 15 units per ml. Excess solution is removed by placing the disk flat against the wall of the tube. A disk saturated in this fashion is placed gently on the surfaces of each of the inoculated plates in the area of the primary inoculum on the left side of the plate. In similar manner a disk saturated with a solution of streptomycin carrying 500 μg. (500 *E. coli* units) per ml. is placed on the right side of the plate (Fig. 1). The disks should be at least 20 mm. apart and on a line parallel to the line of streak so that the inoculum around both disks is comparable. The recommended concentration of the solutions of penicillin and streptomycin were adopted

absence of growth around the disk, the larger the zone the greater the susceptibility. These zones are generally well defined and the diameter is readily measured with a millimeter ruler.

By comparing the size of the inhibition zone with the results obtained by streaking the same organism on the surface of agar containing known concentrations of antibiotic, it was possible to evaluate zone size in terms of units. It was convenient, furthermore, to employ the relative terms, "very susceptible," "moderately susceptible" and "resistant." The data on which these evaluations were made appear in Table 1.

Comment. The penicillin and streptomycin susceptibilities of the significant organisms encountered in 54 selected clinical specimens as determined by the disk method is presented in Table 2. In gen-

eral the results have been very satisfactory. The susceptibilities as determined by this method compare well with those obtained by more sensitive methods.[3,11] It is not the intention of the authors, however, that this method should replace the more sensitive methods. It is designed as a routine test and as a guide in therapy. It may be carried out in any hospital laboratory. The rather broad limits of susceptibility by this method are believed to be sufficiently accurate for the purpose.

In determining criteria of susceptibility the authors were guided by blood level concentrations of both agents which are usually attained by recommended dosages.[9] Concentrations of penicillin and streptomycin greater than 0.5 units and 25 μg. per ml. of blood respectively are difficult to maintain for prolonged periods of time. It is not to be expected that infections caused by organisms with sensitivities of this magnitude or greater will respond to penicillin and streptomycin therapy.

Certain precautions are recommended in carrying out the test. Whereas the solution of streptomycin is quite stable and may be used for several weeks if stored in the refrigerator, the solution of penicillin should be prepared at least once a week and likewise stored in the refrigerator. Solutions of both agents in the concentrations recommended are best prepared in N/15 phosphate buffer, pH 6 for penicillin and pH 8 for streptomycin. Disks should be placed in comparable positions with respect to inoculum. The amount of growth naturally influences zone size, although slight variations in zone size do not materially affect final evaluation of relative susceptibility. Occasionally, heavy growth of a resistant organism may mask zones of inhibition of susceptible organisms. In such instances it may be necessary to subculture the latter organisms to fresh plates and repeat the tests. Inocula from specimens from the upper respiratory tract or from the intestinal tract where a large normal flora is generally present should be kept to a minimum to prevent overgrowth by such resistant organisms.

Occasionally, one encounters within a zone of inhibition a colony or colonies of a susceptible organism. The authors have considered such colonies resistant variants. The production of these resistant forms by an organism which appears to be susceptible may be of great importance in view of the recent reports concerning the mechanism of development of resistance to penicillin[4] and streptomycin.[8] In evaluating the susceptibility of such a culture, the possibility of resistant variants being produced should be taken into account.

Streptomycin differs from penicillin in that it diffuses poorly and is adversely affected by certain ingredients in culture media.[2,6] In an attempt to offset the smaller zones produced by streptomycin, a high concentration (500 μg. per ml.) of that agent was employed. Moreover, since the activity of streptomycin is reduced under anaërobic conditions,[2,6] zones of inhibition on anaërobic plates are either absent or smaller than those on the aërobic plates. It is recommended, therefore, that only the aërobic plate be used in evaluating susceptibility to this agent. This is not true of penicillin; the zones produced by this agent are similar on both plates.

This method may be used readily for testing susceptibility to any antibacterial agent which is water-soluble and diffusible in agar. Susceptibility to tyrothricin was attempted by this method but was unsuccessful because of its insolubility. Morley[12] recently described a test similar to that of the authors in which determinations of susceptibility to sulfathiazole as well as penicillin were attempted. As a whole the results with sulfathiazole were poor and did not appear to warrant use of this method for the routine testing of susceptibility to sulfonamides.

Aside from its value in determining susceptibility, the routine use of this test assists in colony identification. With a general knowledge of the relative suscep-

tibility of the important pathogens to penicillin and streptomycin, one is often guided by the zones of inhibition in identifying colonies of *H. influenzæ* and other bacterial types.

Summary. A simple method is described for determining susceptibility to penicillin and other antibiotics at the time of primary isolation. Its essential feature is the placing of filter paper disks saturated with antibiotic solution on the surface of a blood agar plate immediately after its inoculation with a clinical specimen. The routine use of this procedure provides valuable assistance in the selection of the proper antibiotic agent.

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THROMBOPHLEBITIS ON THE MEDICAL SERVICE OF A GENERAL HOSPITAL

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The subject of thrombosis and embolism has received increased attention in the surgical literature during recent years. This has resulted from the recognition of the condition of quiet deep venous thrombosis originating in the foot and calf (the "bland thrombosis" of Homans and the "phlebothrombosis" of Ochsner) and the introduction of surgical treatment in the form of proximal ligation and thrombectomy.

The frequency of thrombophlebitis complicating medical illness is often not fully appreciated by the internist, and consequently this complication is all too frequently unrecognized during its early stages. Likewise, proper preventive measures are often neglected. Constant vigilance must be maintained to arrive at early diagnosis, upon which depends the prevention of pulmonary embolism and limitation of disability from residual venous obstruction. During the first 18 months of operation of this General Hospital on the Assam-Burma border, thrombophlebitis was rarely diagnosed on the Medical Service. In recent months the occurrence of pulmonary embolism in several patients with scrub typhus has directed our attention to the frequence and importance of deep venous thrombosis. During the past 4 months (Nov. '44 to Feb. '45), thrombophlebitis has been diagnosed 10 times in 9 patients. These cases have been analyzed and the problem of thrombophlebitis in general is discussed with reference to the more recent reports.

Material. There were 10 instances of thrombophlebitis occurring in 9 patients, all of whom were American soldiers. Eight of these developed in patients with scrub typhus during the management of 130 consecutive cases of this disease—an incidence of 5%. One developed in a patient suffering from a depressive psychosis and one in a patient with acute infectious arthritis.

All occurred in the lower extremity. There were 6 instances of primary involvement of calf veins, in 2 of which the process extended into the femoral veins (Cases 1 to 5). There were 2 instances in which the thrombophlebitis apparently originated in the pelvic (ilio-femoral) veins (Cases 8 & 9). In 2 patients the site of origin could not be accurately determined, since the process involved the whole venous system of the leg from calf to pelvis at the time of recognition (Cases 6 & 7). A brief summary of each case is given in Table I.

Diagnosis. The early recognition of thrombophlebitis complicating an illness depends upon the alertness of the physician and a knowledge of the early manifestations of such a lesion.

(1) *Thrombophlebitis limited to the calf veins.* Symptoms and signs were present quite early in our cases of deep calf vein thrombosis. In all 6 cases attention was directed to the lesion by the complaints of discomfort and stiffness in the affected calf. Such mild symptoms do not always evoke spontaneous complaint from a patient whose attention is centered upon other more distressing ailments. In two instances specific questioning was necessary to ascertain the presence of calf discomfort. Homans' sign (increased pain in the calf on passive dorsi-flexion of the foot associated with tenseness to palpation) was present in all cases at the time symptoms were first noted. Appreciable swelling and vasomotor, color, and temper-

ature changes in the lower leg were absent as long as there was free flow of blood through the femoral vein. Fever, usually not exceeding 99.5° F., was present.

(2) *Thrombophlebitis limited to the calf and femoral veins.* Involvement of the femoral vein manifested itself by signs of (1) inflammation (pain and tenderness) along the course of that vessel and (2) obstruction to venous return in the lower leg (superficial venous dilatation, warmth, cyanosis, and swelling below the knee). There was usually low-grade fever not exceeding 100.5° F. but the systemic reaction was mild. It should be emphasized here that these findings may be absent while a long propagating thrombus, attached only in the calf, floats free within

TABLE 1.

Pt.	Primary disease and Site of 'phlebitis	Symptoms and signs	Treatment	Course and results
1.	Scrub typhus. (a) R. calf veins. (b) L. calf and femoral veins.	(a) Calf pain, tenderness, and positive Homans' sign noted 14th day of illness.	(a) None.	(a) Therapy neglected due to failure to diagnose the lesion. No embolism.
		(b) Calf pain, tenderness, and positive Homans' sign; pain and tenderness over femoral vein; warmth, cyanosis, superficial venous dilatation, swelling of calf; leg held rigidly semi-flexed; fever 100°; noted 42nd day of illness.	(b) Bandage, elevation and exercise. Ligation L. fem. vein, 43rd day. P-V injection 43rd, 44th, and 45th days.	(b) P-V injection necessary for relief of pain following ligation. Ambulatory on 47th day. No embolism. Residual disability requiring disposition to U. S. A.
2.	Scrub typhus. R. calf veins.	Calf pain, tenderness, and positive Homans' sign noted 15th day of illness.	Bandage, elevation, and exercise. P-V injections 15th, 16th and 18th days.	Good results. Ambulatory on the 16th day. No embolism. Returned to duty.
3.	Scrub typhus. R. calf veins.	Calf pain, tenderness, and positive Homans' sign noted 22nd day of illness.	Bandage, elevation, and exercise. P-V injection 33rd day.	During 10 days of bandage, elevation, and exercise, there was neither extension or improvement. Excellent result following a single P-V injection. No embolism. Returned to duty.
4.	Scrub typhus. R. calf and femoral veins.	Pain rt. calf on 16th day of illness, not mentioned until 19th day. Pain, tenderness, and positive Homans' sign with moderate swelling of the calf present on 19th day. No signs over femoral vein.	Bandage, elevation, and exercise. P-V injection 19th day. Femoral vein ligation 19th day.	Severe pulmonary embolism 8 hours after the P-V injection. Ligation following the embolism. Long illness with residual disability in right leg requiring disposition to U.S.A.
5.	Acute arthritis, right ankle. R. calf veins.	Calf pain, tenderness, and positive Homans' sign noted 8th day of immobilization.	Bandage, elevation and exercise. P-V injection 8th day immobilization.	Immediate relief of symptoms and signs. No embolism. Returned to duty after arthritis subsided.
6.	Scrub typhus. L. ilio-femoral veins.	Unrecognized until patient had been ambulatory for 45 days. Then swelling and discomfort without signs of activity.	Bandage, elevation, and exercise.	No embolism. Residual disability in left leg required disposition to U. S. A.
7.	Psychosis. L. calf and ilio-femoral veins.	At least 2 episodes of pulmonary embolism before 'phlebitis was diagnosed. Calf pain, tenderness, and positive Homans' sign; pain and tenderness over the femoral vein and in the groin; warmth, cyanosis, superficial venous dilatation and marked swelling of the whole limb; decreased pulsation of femoral artery.	Bandage, elevation, and exercise. Continuous I-V Heparin 11 days.	Prompt subsidence of fever, symptoms, and signs. No further embolism. Febrile reaction to heparin 11th day. Residual disability sufficient to require disposition to U. S. A., even had this not been necessary due to the psychosis.
8.	Scrub typhus. (a) L. ilio-femoral veins.	(a) Unexplained recurrence of fever of 103° 25th day illness. Signs of moderately advanced ilio-femoral thrombophlebitis 32nd day.	(a) Bandage, elevation and exercise. Continuous I-V heparin 6 days.	(a) Prompt subsidence of fever, symptoms, and signs. Ambulatory on 4th day of heparin. No embolism. Disability sufficient for disposition to U. S. A.
	(b) L. calf veins.	(b) Signs of acute calf vein thrombophlebitis 43rd day —3 days after heparin discontinued.	(b) P-V injection.	(b) Prompt and permanent relief of symptoms. No embolism.
9.	Scrub typhus. L. ilio-femoral veins.	Unexplained recurrence of fever to 103° on 30th day of illness. Signs of moderately advanced ilio-femoral thrombophlebitis on 36th day. Calf signs absent.	Bandage, elevation, and exercise. Continuous I-V heparin 8 days.	Prompt subsidence of fever, symptoms, and signs. No embolism. Residual disability sufficient to require disposition to U. S. A.

the lumen of the femoral vein if the wall of that vein is not actually involved in the process.

(3) *Ilio-femoral thrombophlebitis.* Frank ilio-femoral thrombophlebitis gave signs of more severe inflammation and obstruction. Rather marked pain and tenderness were present in the lower abdomen and femoral triangle. Swelling of the whole extremity from groin to foot was present in addition to heat, cyanosis, and superficial venous dilatation. In the upper thigh, the sulcus medial to the femoral artery, occupied normally by a compressible vein, was obliterated and the femoral arterial pulsation was reduced. Fever was present, intermittent in character and often reaching 104° F.

Cases 8 and 9 were instructive. These two patients were afebrile, ambulatory and convalescing satisfactorily from scrub typhus when a secondary rise in temperature occurred. As no other cause for fever was found, they were carefully examined daily for signs of a suspected thrombophlebitis and none was found for several days. Then suddenly in both of these patients, the findings of a moderately advanced ilio-femoral thrombophlebitis appeared. Even then signs of calf vein involvement were absent.

Treatment. For therapeutic purposes, our patients were divided into 3 groups: (1) those with signs of calf vein involvement only, (2) those with signs of both calf and femoral vein involvement, and (3) those with frank ilio-femoral thrombophlebitis.

In the first group, treatment consisted primarily of lumbar paravertebral procaine injections. We believe that this method is safe if one can be sure that the lesion is early and that no thrombus is present in the femoral vein.

For the second group, we employed ligation of the femoral vein. Lumbar paravertebral procaine injections were used only for the relief of pain after ligation.

The third group was treated with heparin, this being the only anticoagulant available to us. Heparin was administered by continuous intravenous drip at a rate sufficient to maintain the clotting time between 30 and 40 minutes. "Bicycle" bed exercises were instituted on the third day of heparinization and the patients were made to walk on the fourth day. The use of paravertebral procaine injection in a patient who is receiving anticoagulant is definitely contra-indicated because of the danger of retro-peritoneal hemorrhage from needle puncture of vein in the paraspinal region.

In all 3 groups elevation, compression bandage, bed exercises, and earliest possible mobilization were used routinely.

Results. (1) *Thrombophlebitis limited to the calf veins.* The results of procaine sympathetic block were satisfactory in 3 patients (Cases 2, 3, 5) with thrombophlebitis limited strictly to the calf. There was prompt symptomatic relief with no extension of the thrombosis after the first injection. A single injection was sufficient in 2 instances, whereas 3 injections at daily intervals were necessary in the other. There was no residual disability and all of these patients were returned to full military duty. The remaining instance of thrombosis limited to the calf veins was untreated because of failure to diagnose the lesion at an early stage.

(2) *Thrombophlebitis limited to the calf and femoral veins.* One patient (Case 4) with calf and femoral vein involvement received a paravertebral procaine injection. Eight hours later this patient suffered a severe pulmonary embolism and the femoral vein was ligated immediately thereafter. In retrospect, this appears to have been an error in judgment. Following the ligation in this case there was permanent relief of pain in the leg. In one other case of calf-femoral vein thrombophlebitis in which the femoral vein was ligated, pain from venous spasm persisted until relieved by lumbar sympathetic procaine block. Both of these patients had residual swelling and disability severe enough to require disposition to the zone of the interior.

(3) *Ilio-femoral thrombophlebitis.* Good results followed heparin therapy in 3 treated cases of ilio-femoral thrombophlebitis (Cases 7, 8, 9). The process quickly subsided and no embolism occurred. In one of these (Case 8), the administration of heparin was discontinued after 6 days. Three days later, signs of acute deep calf vein thrombophlebitis appeared. These signs subsided rapidly after one lumbar paravertebral injection of procaine. There was one febrile reaction apparently due to heparin. This patient experienced a chill followed by fever of 104° F. for which no other cause could be found. The fever disappeared within a few hours following the discontinuance of herapin. The remaining case of ilio-femoral thrombophlebitis (Case 6) was untreated since it was quiescent when discovered. All of these patients had rather marked residual disability necessitating evacuation to the zone of the interior.

Comment. Considerable advances have been made in recent in the understanding of the fundamental problems of thrombophlebitis. Recognition of the fact that the pelvic veins are not the usual primary site of thrombophlebitis is of major importance. While the majority of thrombotic processes originate in the foot and calf veins, the pelvic veins are occasionally the initiating site.[18] Observation of cases 8 and 9 supports this view.

The term "phlebothrombosis" has become used to differentiate between supposedly non-inflammatory venous thrombosis and frankly inflammatory ilio-femoral thrombophlebitis or phlegmasia alba dolens. It may be that this concept is not strictly accurate. Except in cases of purely traumatic origin, it appears that all venous thrombosis is associated with inflammation of the vein wall, that associated with phlebothrombosis differing only in degree from that in ilio-femoral thrombophlebitis. Jensen[15] has called attention to the presence of microscopic inflammatory changes in sections of femoral veins removed at operation from cases of so-called phlebothrombosis.

Many cases of pulmonary embolism occur before the site of the thrombus has been detected. Thrombosis originating in the calf with a long, unattached, non-obstructive, centrally propagating thrombus is responsible for the majority of these cases.[14] With constant vigilance on the part of the physician, many of these instances of calf vein thrombosis may be detected in time to prevent pulmonary embolism. When the picture of ilio-femoral thrombophlebitis has become manifest, there is much less danger of embolism; then obstruction to the lumen is complete, the thrombus is firmly attached, and the likelihood of a detachable thrombus extending beyond the junction of the external and internal iliac veins or of the two common iliacs is not great.

There have been three major therapeutic approaches to the problem of thrombophlebitis: (a) anticoagulant therapy, (b) sympathetic block by use of lumbar paravertebral procaine injections, and (c) surgical treatment by ligation of the vein above the thrombus with or without thrombectomy.

Following its introduction in 1938 by Murray and Best,[19] heparin has been rather widely used to increase the coagulation time of the blood. Its utility has however been somewhat limited by difficulty of constant intravenous administration and expense. More recently Loewe and Rosenblatt[17] have reported the use of heparin-Pitkin menstruum subcutaneously. Subsequently Loewe, Rosenblatt, and Hirsch[18] have reported good results in treatment of 125 cases of thrombophlebitis of various types with heparin-Pitkin menstruum. Bauer[7] from Sweden has reported enthusiastically on the efficacy of heparin in preventing extension of thrombosis. During a 5 year period while intermittent intravenous heparinization was used, only 3 fatalities from embolism occurred in 209 cases of deep thrombosis, as compared with 47 embolic deaths in 264 cases of thrombosis before heparin therapy was used. One of his deaths occurred before heparin was administered. One occurred

from fresh thrombosis in the opposite leg after discontinuance of heparin, the patient having not been allowed out of bed. He calls attention to the importance of mobilizing patients before heparin is discontinued. Most of the heparin failures have resulted from allowing the patients to remain in bed after the anticoagulant was discontinued.

Because of the ease of oral administration, dicoumarol has become popular as a means of delaying blood clotting. However its use is limited by the necessity of having laboratory facilities available for the daily determination of blood prothrombin levels. Dicoumarol has been extensively used by Barker and his associates[4,5,6] in both prophylaxis against and treatment of thrombo-embolism. In a group of 138 cases of postoperative thrombophlebitis treated with dicoumarol, they had no fatal embolism and only 4 patients developed subsequent episodes of thrombophlebitis.[6] Yahr *et al.*[26] have also recently reported good results in 57 cases of thrombophlebitis of various types treated with dicoumarol.

The lumbar sympathetic block was introduced by Leriche[16] and popularized in this country by Ochsner and DeBakey.[20,12,22] Good results, both immediate and permanent, have been cited by these and other authors from the use of this procedure in various types of thrombophlebitis. However Ochsner[23] has recently advocated that use of this procedure be reserved primarily for relief of vasospasm associated with ilio-femoral thrombophlebitis.

Interruption of the venous channels as a means of treating thrombophlebitis was initiated by Homans in 1937.[13] Since then the trend has been toward more frequent use of vein ligation and thrombectomy. There are many reports of good results following surgical treatment of this nature.[1,2,3,8,10,12,14,15,23,24,25] It has become common practice to ligate both femoral veins in the attempt to prevent further embolism even though signs of thrombophlebitis may be limited to one leg. Homans[14] has advocated the use of iliac or vena caval ligation in cases of repeated embolism. Some have advocated the use of anticoagulants in conjunction with interruption of the venous channels and (or) thrombectomy.[2,15,24]

Even so, it remains apparent that no one of the procedures at use at the present time is adequate for the management of all forms of venous thrombosis or for the prevention of embolism. Unexplained failures have been noted with dicoumarol[9] and embolism has occurred following the use of heparin.[4,15] We have had a case of embolism following the use of sympathetic block. Six per cent of patients who have had embolism prior to vein ligation have a repetition of embolism following this procedure.[2] Dennis[11] has reported a case of severe ischemia following femoral vein ligation. The patient almost lost his leg and was still unable to walk on it 6 months later. This author strikes a note of warning and conservatism regarding indiscriminate vein ligation. One has to admit, then, that we have no perfect method with which to combat thrombo-embolism at the present time.

It has seemed to us that the indications for the use of one or more of the major forms of therapy differ considerably depending upon such factors as the site of origin of the thrombotic process, its duration and extent, and whether or not embolism has already occurred. By using sympathetic procaine block therapy for the group with thrombophlebitis limited to the calf, we were able to return this group to full military duty. Had femoral vein ligation been employed, disability sufficient to prevent return to full duty would have resulted. The use of sympathetic block for this sort of case is said by many to be dangerous and we emphasize that one should be extremely careful to select for this procedure only patients in whom the process is known to be early and in whom there are no signs to indicate thrombosis above the knee. Barker *et al.*[6] state that there is evidence that when thrombophlebitis is diagnosed clinically, the thrombus which is present will almost

certainly not detach and become an embolus. The use of sympathetic block would seem particularly suited to the patient who develops calf thrombophlebitis after he has become ambulatory. The danger of embolism should be small indeed and they are spared the "minimal" immediate disability and the undetermined amount of permanent disability resultant to femoral vein interruption. In the one case in which embolism followed sympathetic block, it is noteworthy that there was clinical evidence of extension of the thrombosis into the femoral vein at the time of the procedure. The danger of embolism is greatest when a propagating thrombus occupies the lumen of the femoral vein. In such instances, it appears safest in spite of the disability which results to remove the clot and ligate the vein, perhaps employing anticoagulants subsequently. In ilio-femoral thrombophlebitis, the danger of embolism is much less and surgical procedures necessarily much more radical. Ligation of the iliac vein or the vena cava is a major surgical procedure which becomes formidable in a patient who is already suffering from a serious disease. Here it would appear that anticoagulant therapy is most useful.

In view of the danger and disability resulting from venous thrombosis, prevention assumes a most important role. An understanding of the factors which contribute to intravascular clotting is essential to any program of prevention. The principal factors in the development of this condition are: (1) decreased rate of blood flow, (2) injury to the endothelium, (3) increased coagulability of the blood, (4) increased vasomotor tone, and (5) the "X" factor—presumed to be of toxic nature, liberated into the circulation as a result of disease, injury, or operation. Factors promoting venous stasis were present in all of the cases presented here. The typhus patients were prostrated and confined to bed. In addition their beds were fitted with Gatch frames which allowed marked flexion of the knees and thighs—thus favoring a certain degree of venous obstruction both at the knee and groin. De Takats[10] has called attention to the Fowler position as a factor in venous stagnation. The psychotic patient lay immobile for long periods of time. The patient with arthritis was treated by immobilization of the affected extremity. With regard to injury to the endothelium, rickettsial diseases in general are known to damage the capillaries and small blood vessels. However, we know of no specific observations concerning the condition of the endothelium of the veins of the legs in scrub typhus. We have no observations concerning an increase in the coagulability of the blood, the vasomotor tone, or the "X" factor in these patients.

Simple prophylactic measures which include (1) elevation of the foot of the bed, (2) early institution of bed exercises, and (3) avoidance of the unnecessary use of the Fowler position, immobilization, and poorly applied constricting bandages should appreciably lower the incidence of thrombophlebitis. In very ill patients, nursing care should include message and passive motion of the lower extremities in addition to the usual backrubs. We believe that the institution of the Army reconditioning program for bed patients as well as for convalescent patients is extremely important in the prophylaxis of thrombophlebitis.

Summary. (1) Ten instances of thrombophlebitis in the lower extremity were observed on the Medical Service of this General Hospital during a four month period.

(2) Eight of these developed in patients with scrub typhus fever.

(3) Early diagnosis was not achieved until we began to look carefully for the early signs.

(4) In our opinion, treatment of thrombophlebitis should be individualized depending on the location and extent of the lesion.

(*a*) Thrombophlebitis confined to the calf veins was treated by lumbar paravertebral sympathetic block with uniformly good results. This procedure prob-

ably tends to prevent central propagation of the thrombus, embolism did not occur, and patients were spared the prolonged disability which follows femoral vein ligation. The use of heparin of dicoumarin in these cases might help prevent propagation of the thrombosis. However, its use is usually unnecessary. The concomitant use of anticoagulant and lumbar sympathetic block is prohibited by the danger of retro-peritoneal hemorrhage.

(*b*) Thrombophlebitis involving the calf and femoral veins was treated by proximal ligation. Sympathetic block was used only for relief of pain after ligation. In one case of calf vein thrombosis with extension into the femoral vein, paravertebral procaine block prior to femoral ligation was followed promptly by pulmonary embolism.

(*c*) Ilio-femoral thrombophlebitis was treated with heparin, high ligation being inadvisable in these ill patients. The results were satisfactory.

(5) This choice of therapeutic methods for the treatment of specific thrombotic lesions seems rational. It has been successful thus far in preventing embolism and in minimizing disability.

(6) In addition to these specific measures, all three groups of patients were treated with elevation of the extremity, compression bandage, bed exercises, and early mobilization.

(7) Our observations suggest that all cases of ilio-femoral thrombophlebitis do not necessarily result from extension of a calf vein thrombosis; such a process may originate occasionally in the ilio-femoral region.

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PLASMA SUBSTITUTES

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The need for an effective colloidal infusion solution without serious side effects stimulated a renewed interest in substitutes for plasma in the early years of the recent war. Could either the duration of the war or the response of the American people to the plea for blood donations have been predicted accurately, the program of investigations into plasma substitutes might not have been so extensive. Had that been the case, we could not have gained the knowledge of the physiologic properties and the physicochemical characteristics of so many substances suggested as plasma substitutes in such a relatively short time.

There is as yet no acceptable substitute for blood if one is to consider its oxygen-carrying function. Plasma has been accepted through necessity as a "blood substitute." The introduction of such materials as pectin, isinglass, methyl cellulose, globin, bovine albumin and gelatin has been for trial as substitutes for plasma rather than for whole blood. It is recognized, however, that the infusion of any colloidal solution which increases the blood volume, augments the cardiac output and is, therefore, a blood substitute in a limited sense.

Many though the functions of plasma may be, the greatest uses for plasma are in the treatment of peripheral vascular collapse and in the maintenance of protein nutrition by the intravenous route. Inasmuch as the recent need was for a colloid to be used in combating shock, the criteria for a plasma substitute became the criteria for an agent for the treatment of circulatory collapse.

The Criteria for an Agent for the Treatment of Shock. Certain minimal specifications for a plasma substitute could be inferred from the physiochemical properties of the serum proteins which are responsible for the colloidal functions of plasma. It does not follow, however, that the properties of a plasma substitute must be identical with those of the serum proteins. By reasoning thus, it becomes easy to think in terms of physiochemical structure rather than in terms of physiologic and clinical response. It

is quite possible that colloids having physiochemical properties at variance with the plasma proteins might prove to have advantages over plasma itself in special circumstances. A knowledge of the molecular structure, however, governing as it does the viscosity and osmotic presure, is of great help in improving and perfecting a plasma substitute.

One of the most significant factors in peripheral vascular collapse, no matter what the cause, is the diminished circulating blood volume. Whole blood is usually the ideal replacement fluid, but there are some varieties of shock and shock-like states when some clinicians prefer a colloidal solution without erythrocytes and therefore use plasma. There are also times when blood is not obtainable, or not obtainable without some delay for blood grouping and cross-matching, or not in sufficient quantity to meet a specific need. In these instances plasma is better for injection than a non-colloidal solution such as isotonic sodium chloride which dilutes the serum proteins and diminishes the colloid osmotic pressure, resulting in a greater and more rapid escape of fluid from the vascular tree.

The proteins of plasma probably are utilized for several functions that one could not expect to find in a single substitute. The chief function, however, at which a substitute is directed is the oncotic one.

An acceptable plasma substitute should satisfy certain minimal physical, physiologic and immunologic criteria. The physical properties of such a substitute are: (1) stability, (2) ease of storage, (3) low viscosity, (4) high osmotic pressure, and (5) a colloidal particle size large enough to be retained in the circulation.

From a physiologic standpoint a plasma substitute must be safe for injection in large quantities, must be retained in the circulation long enough to be effective as an oncotic force, and should not interfere with the function of internal organs. Of these last functions attention should be turned especially toward blood coagulation, the defense against infection, tissue repair, and the function of liver and kidneys.

Immunologically a plasma substitute should not be an antigen, or at most should be a poor one. If any antigenicity can be demonstrated, it should not be associated with any sensitivity to horse or rabbit serum. Any material intended for a plasma substitute should have a minimum of natural sensitivity and no associated food sensitivity.

Gum Acacia. Gum acacia is the one colloidal infusion material remembered from the first World War. This polysaccharide was used extensively until study of its toxicity, especially with reference to its antigenicity and its tendency to produce liver dysfunction and hypoproteinemia, placed it in disfavor.[15,17,21,22,93]

In spite of its untoward side effects, solutions of gum acacia were good substitutes for the oncotic functions of plasma, and many investigations into its properties have provided the pattern for study of later substitutes.

Human Serum Albumin. One of the substitutes for plasma which was studied most extensively was human serum albumin. Being derived from plasma itself it is not a true plasma substitute in its sparing effect on blood donations. At the same time, since it is a fraction of plasma, it is subject to less criticism than artificial substitutes.

Safe and effective though human serum albumin may be, it was prepared originally for military use with mobile forces[60,90,91] with relatively little regard to cost. Unless cheaper methods of fractionation can be achieved or the cost of human serum albumin subsidized, it seems improbable that it will enjoy wide clinical use in civilian medicine.

Serum albumin has a molecular weight of 69,000 (plasma protein average 90,000) and on a theoretical basis should hold 18 cc. of fluid in the blood stream per gram.[73] This theoretical measure of oncotic function has been confirmed by determinations of plasma volume increase

after infusion of the human serum albumin.[9,45,87]

Albumin solutions have a high degree of thermal stability[74] and may be given intravenously with a very low reaction rate. Globulin deficiency after repeated infusions has been reported.[37] Following the injections of serum albumin there is a hemodilution which is well sustained in depleted subjects but which is transient in normal controls. Such hemodilution and plasma volume increase is most marked if additional fluids are given with the albumin. Fluids should be given with albumin infusion in dehydrated patients.[35]

Serum albumin is well retained if there is no hemorrhage or loss through burned surfaces.[9] Infusions of albumin are effective in the treatment of shock and burns and are followed by an increase in right auricular pressure, arterial pressure and cardiac output.[9,37,87]

Albumin is utilized like native serum protein, but only a small portion is retained in the circulation after injection, the remainder evidently being stored.[37] Hypoproteinemia may be corrected if sufficiently large amounts are used.[35] Study of tissues following repeated infusion reveals no evidence of pathologic change.[37]

Albumin has been used in the treatment of the nephrotic state; and while there is a definite increase in proteinuria following infusion, there is not always an accompanying diuresis.[35,37] There is but temporary improvement in cirrhotics following infusion of human serum albumin.[37] A salt-free preparation has been made available which is a better agent, theoretically at least, for the mobilization of edema.[75]

Gelatin. Gelatin solutions were used first as a plasma substitute by Hogan in 1915,[28] but were abandoned for several reasons. Bayliss,[4] who had done much to establish gum acacia as an effective agent for the treatment of shock, objected to gelatin on the theoretical ground that all contaminating spores might not be killed in its preparation. Other investigators used methods of preparation of solutions at variance with Hogan's and obtained different results which did much to discredit the earlier work.

Between the 2 wars gelatin was mentioned as a substitute for plasma only once. Wolfson and Teller,[92] using the refractive index of serum, reported that infused gelatin was retained in the circulation long enough to be effective in posthemorrhagic hypotension.

The more recent investigations into the suitability of gelatin as a plasma substitute indicated that it was both safe and effective as a substitute for the oncotic function of plasma in the treatment of shock and hemorrhage. Inasmuch as gelatin solutions are composed of molecular aggregates of varied size the question of optimum molecular structure became an important one. It was apparent that no gelatin would approximate closely the molecular configurationof the native plasma proteins so that the choice of a gelatin solution for use as a plasma substitute lay between a long and a short molecular chain structure.

The viscosity, gel strength, modulus of rigidity and temperature of solidification are directly proportional to the size of the molecular chain while the fluidity and melting point are inversely proportional to molecular chain size. The small molecules, while having a higher theoretical osmotic pressure than the large molecules, are lost rapidly through the kidneys so that the effective osmotic pressure is actually higher with the large molecular gelatin. The best preparations from the standpoint of physiologic response then have the practical disadvantage of high viscosity.

Gelatins of any molecular structure seem to be innocuous and safe for injection in large quantities.[27,34,40,56] The absence of liver and kidney damage in both dogs and patients after infusion of gelatin has been demonstrated.[40,47,56,63] More rigorous tests were used by Van Slyke[85] in which gelatin was given to animals

with renal ischemia. Recovery was as rapid and regular in the gelatin infused animals as in the controls. Morphologic changes found in animals after massive gelatin infusion and after repeated infusions in all instances were reversible.[68] Human autopsy material revealed no changes attributable to gelatin.[42] Untoward reactions following gelatin infusion in human subjects are infrequent,[44] and the venous thrombosis found after some gelatin infusions was demonstrated to be due to a mercurial preservative.[65]

Following the injection of gelatin intravenously there is an immediate rise in serum gelatin concentration and a concomitant fall in native serum proteins. The serum gelatin concentration diminishes gradually thereafter with a reciprocal rise in serum protein concentration. The speed of this reciprocal reaction is inversely proportional to the molecular size of the gelatin while the duration of detectable gelatin concentration in the blood is directly proportional to the molecular size.[27,40,47,53,63]

Highly degraded (short molecule) gelatin may leave the circulation completely in less than 24 hours while minimally degraded varieties (long molecules) disappear completely only at the end of 5 days.[27,40]

Blood coagulation is not effected following gelatin infusion,[40,47] and although the hematocrit reading and the hemoglobin concentration are decreased, the total red cell volume is not diminished either in animals in shock or in normal unanesthetized controls.[53,55] The sedimentation rate is increased markedly after gelatin infusion according to all observers.

The increased sedimentation rate seen with so many of the macromolecular colloidal substitutes for plasma is a measure of the degree of pseudo-agglutination of the erythrocytes. The phenomenon of pseudo-agglutination is apparently without deleterious effect insofar as gelatin is concerned, based on observations of oxygen transport[62,63] and direct visualization of capillaries after gelatin infusion through a rabbit's ear window.[1,63]

The possibility of difficulty with the cross-matching of the blood of patients recently infused with gelatin (up to 4 days) was eliminated by the utilization of glycine to prevent the phenomenon of pseudo-agglutination in serum cell suspensions.[43]

The use of various gelatin solutions in the treatment of experimental shock revealed differences resulting from the type of gelatin preparation used, but, in general, gelatin was found to be an effective agent for the restitution of colloidal osmotic pressure. Swingle and his associates[39,80,81] found gelatin to be superior to saline and demonstrated pooled heparinized plasma to be no better than gelatin. Parkins'[62] work indicated that gelatin is among the best of the colloidal substitutes for plasma. Gordon, Hoge and Lawson[20] found gelatin to occupy an intermediate place between blood and crystalloids in its ability to maintain the circulation after blood loss. Winkler, Danowski and Elkington[89] found gelatin to be equal to serum in the treatment of salt depletion shock.

Attention has been called to the differences between gelatin types and molecular structure by several groups of investigators.[27,38,40,41,56] Clinical superiority was claimed definitely for the long molecular structures by most observers,[58,59] although it was recognized that the short molecular form was both effective and innocuous.

The excellent study from Whipple's[72] laboratory revealed that gelatin caused no alarming disturbances; it produced both plasma protein and hemoglobin when given by mouth, produced blood protein equivocally when given by vein, and had no inhibitory action on the formation of plasma proteins from casein hydrolysates or amino acids when those materials were infused after gelatin. These studies were done with doubly depleted dogs (anemic and hypoproteinemic), and on the basis of the behavior

of these animals 2 questions were raised: the possibility of toxicity from gelatin when given over a period of 1 to 2 weeks and the possibility of gelatin infusion causing further damage in kidneys already diseased. The prolonged administration of gelatin is apparently without deleterious effect in patients,[27,40,42,56] and it does not seem to alter kidney function adversely in the diseased or damaged kidney.[27,40,42,44]

In normal individuals the administration of poorly degraded gelatin solutions is attended by an increase in cardiac output and by an increase in plasma volume, but not by a rise in arterial pressure.[19,40] In the patient in peripheral collapse the same physiologic changes are seen, but the low blood pressure is rapidly returned to normal. In the shocked individual with depleted plasma volume 52 gm. of gelatin (P–20) in 1000 cc. of fluid increased the plasma volume for 4 to 6 hours to the same extent as did 50 gm. of human serum albumin.[34,38,40,71] Plasma volume increases are greater but less sustained following gelatin infusion as compared with blood and plasma.[42,71]

Poorly degraded gelatin solutions have been used as an agent for the mobilization of edema with variable results.[6,44] The use of highly degraded (short molecule) gelatin in edematous states is not without danger because of the escape of small gelatin particles into the edema fluid.[42] The larger gelatin molecules (P–20) pass through purposely injured capillaries only 35 to 60% as much as do the plasma proteins.[55]

Of all the plasma substitutes, except those derived from plasma itself, gelatin is the only protein. Even though it is an incomplete protein, it is at least partially metabolized. Brunschwig[7] demonstrated the metabolism of gelatin in animals and man,[7] and Koop and his associates[46] found that when nitrogen is supplied as half gelatin and half casein or fibrin hydrolysate, the utilization of nitrogen was better than that achieved with the hydrolysate alone.[46]

Two variations of gelatin solutions have been prepared as plasma substitutes: isinglass (icthyocolla) and oxypolygelatin.

Isinglass, a collagen prepared from the swim bladders of hake with 96.6% of its protein available as amino acids[5] was studied by Taylor and his associates.[84] After animal experiments and clinical trial it was thought that a suitable animal gelatin rather than isinglass offered greater assurance of effective maintenance of blood pressure because of the much longer time it remained in the blood stream of the infused animal.[88]

Oxypolygelatin, an oxidized, polymerized gelatin was introduced by Pauling[66] and developed to the point of reproducibility. Observations by Parkins[62] indicate that the application of oxidation and polymerization procedures to ossein gelatin might increase its fluidity without serious change in its physiologic characteristics.

Bovine Albumin. Whole bovine plasma has been known to produce alarming reactions when given intravenously to other species.[86] Such reactions were associated with the globulin fraction of bovine plasma to a large degree.[50] Bovine albumin can be prepared in such fashion as to have a low reaction rate[83] and a nonbacterial chill-producing substance has been described in bovine albumin.[49] Reactions with the purified albumin when they do occur include urticaria, diarrhea, fever, nausea, vomiting and chill.[83]

The bovine albumin is a potent antigen,[24,32,52,82] and there is the possibility of cross-reaction with beef products taken by mouth.[25] In spite of this it has been administered to patients.[10,16,36]

The infusion of a bovine albumin to animals in thermal shock is a therapeutic procedure not attended by toxic reactions but less effective than plasma when large and continuous protein and fluid losses are encountered.[13] The plasma volume and circulatory tone are adequately maintained after infusion, and the ability of bovine albumin to hold

fluid is equal to that of human serum albumin.[26]

Serum and urinary potassium are not significantly altered after infusion. The morphologic changes produced in rabbits following infusion of bovine albumin are all reversible, but this does not carry over to human subjects necessarily.[3]

Globin. A modified globin prepared from the erythrocytes of man has been used by Strumia and his co-workers[79] as a substitute for the colloidal osmotic functions of plasma. A preliminary report published by them in 1945 indicated that globin had these general attributes: (1) solubility in water at pH 7.4, (2) minimum solubility at pH 6.5, (3) a viscosity lower than that of citrated plasma, (4) a molecular weight of 85% of the globin equal to 34,000, (5) a symmetrical molecule, (6) an approximate yield of 250 gm. per 1000 cc. of packed erythrocytes, (7) stability in solutions of 0.85% saline (under ordinary conditions of preservation), (8) miscibility with citrated blood or plasma, (9) failure to increase erythrocyte sedimentation rate, and (10) production of hemodilution lasting 24 to 120 hours after intravenous infusion.

The authors found no evidence of antigenicity or toxic properties other than pyrogenic reactions and what they called a "pressor effect" and a "vasomotor effect." Globins exhibiting instability at pH 7.4 were not used in clinical investigations because of the embolic phenomena associated with death and organic damage encountered in animals when such globins were used.

The globin prepared by Strumia and his co-workers[77,78,79] was reported by them to be satisfactory in the treatment of shock from trauma, hemorrhage and burns, in the correction of hypoproteinemia and in the treatment of edema in a patient with chronic glomerulonephritis.

Seegal[76] and his associates found human globin anaphylactogenic in the guinea pig. They found no reaction following human globin infusion in mice and rabbits, but dogs exhibited untoward reactions characterized by erythema, urticaria, facial edema, dyspnea, hyperperistalsis, defecation and unsteady gait. Histologic section showed deposits of globin-like material in the proximal convoluted tubules of mice, rabbits and 1 dog within 2 days after injection which were not seen in animals sacrificed 8 days after injection.

Rhoads and Parkins[69] and their coworkers confirmed the results of Seegal and extended observations to include the tolerance of rats to rat globin and dogs to dog globin. They concluded that the efficacy of human and dog globin in treatment of hemorrhage in dogs, and rat globin in burn shock in rats showed globin to be less effective than saline. On the basis of these results observations were not extended to human subjects.[70]

Hemoglobin. The early attempts at preparing a hemoglobin solution free from the stromata of erythrocytes resulted in an infusion material which elicited untoward reactions when given intravenously to animals.[2,61] One of the most serious effects, the blockage of renal tubular lumina by hemoglobinous pigments, was eliminated by improved methods of preparation. Pure hemoglobin solutions when given intravenously are not attended by renal tubular changes[11,12,61] if there is no preëxisting tubular damage.[94]

Solutions of pure hemoglobin carry oxygen and maintain colloidal osmotic pressure for periods up to 36 hours after which they become ineffective because of transformation of hemoglobin to methemoglobin and the loss of injected hemoglobin through the kidneys.[29,50] The erythrocyte sedimentation rate is increased after injection of hemoglobin. A hemoclastic crisis is seen soon after injection, and this is followed by a leukocytosis in 24 hours. No casts are found in the kidney, but deposits of hemoglobinous pigment can be seen in the liver and spleen.[29]

Following the injection of pure hemoglobin solutions into the circulation of dogs there is a rise in blood pressure which cannot be accounted for on the basis of the

oncotic pressure of the hemoglobin solution alone. There is a 100% increase in oxygen consumption following hemoglobin infusion.[51]

Pectin. Solutions of pectin, a polysaccharide, were introduced as a substitute for plasma by Hartman and his associates in 1941.[23] Intravenous injection of pectin in animals and man was followed by no visible deleterious effects and it was non-antigenic.[18] Bryant, Palmer and Joseph[8] reported that intravenous infusions of pectin produced no changes in viscera of rabbits and that none was detectable in the blood 7 days after injection. Hueper,[30] on the other hand, refuted these findings by demonstrating in both animals and man that even partially degraded pectin is retained in liver, kidney, bone marrow, spleen and arteries. Histologic changes included foam cellular formations, hyaline necrosis, foreign body giant cells and calcium incrustations.

Following the intravenous administration of pectin in normal human subjects, the sedimentation rate is increased; the hematocrit falls more than the hemoglobin concentrations; the albumin-globulin ratio is unaffected and the arterial and venous pressure is slightly increased.[48] Plasma volume increases persist 4 hours after infusion.[33]

On the basis of animal experiments, Middleton and Wiggers believed that the usefulness of pectin was limited to the early period of posthemorrhagic hypotension. They found no correlation between hemodilution and favorable response which suggested a possible deleterious influence when used after severe hemorrhage.[57]

As a substitute for plasma, pectin was found to be inferior to serum,[18] less safe than gelatin,[66] and no better than saline in posthemorrhagic prolonged hypotension.[14]

Methyl Cellulose. Like polyvinal alcohol, methyl cellulose has not enjoyed wide use as a plasma substitute. The material that has been given trial is a methyl ether of cellulose and consists of long chains of dextrose molecules with a characteristic high viscosity and a molecular weight of about 50,000. It is effective in shock in animals and is non-antigenic but exhibits the phenomenon of excessive storage in the viscera.[3,31]

Summary. Of the plasma substitutes studied extensively during the recent war years only human serum albumin and gelatin solutions seem to be both safe and effective on the basis of reports available at this time. Human serum albumin derived from plasma itself, is not, strictly speaking, a substitute. Gelatin, in addition to being safe and effective, is also cheap, and when fortfieid with amino acids or protein hydrolysates is a readily available source of parenteral protein.

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OPHTHALMOLOGY

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EXOPHTHALMOS OF ENDOCRINE ORIGIN

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THE concept of the mechanism of production of exophthalmos, with particular reference to thyroid disorders, has not yet reached the stage of universal agreement. There have, however, been significant changes and developments in the past few years and this seems an opportune time to review these changes. Three important articles have been published in the past 2 years which endeavor to correlate our present knowledge, both clinical and experimental, of the production of exophthalmos. There are 2 groups of cases which present different clinical features but which, with advancement of our knowledge, seem to have a single etiologic basis. In the average case of so-called exophthalmic goiter the eye changes are not of particular moment or concern. Much of the change in appearance and of the subjective ocular symptoms is the result of retraction of the lids rather than actual protrusion of the globe. This is true particularly in individuals with other manifestations of definite hyperthyroidism. However, in certain other individuals with thyroid disease, exophthalmos develops progressively which may lead to extreme protrusion of the globe, chemosis of the conjunctiva, inability to close the lids, resultant exposure-ulceration of the cornea, and at times loss of the eye. These cases occur in individuals with both hyper- and hypothyroidism, the extremes ranging from severe thyrotoxicosis to frank myxedema. This latter group of cases with exophthalmos has had a variety of terms applied to it: among the most commonly used are malignant exophthalmos, progressive exophthalmos, exophthalmic ophthalmoplegia, postoperative progressive exophthalmos, special ophthalmopathic type of Graves' disease, and thyrotropic exophthalmos. The reasons for placing this group apart from simple exophthalmic goiter are many. There is a different age and sex relationship, a difference in response to treatment and different findings in the orbit. Exophthalmic goiter affects young adults chiefly and is more common in women than in men. Progressive exophthalmos affects middle aged adults and men are more common in this group than women according to reports by Mulvany.[9] The urine of patients with ordinary exophthalmic goiter shows little or no thyrotropic activity while that of patients with progressive exophthalmos contains an excessive amount of thyrotropic substance. In general, the proptosis and lid retraction of exophthalmic goiter recedes following thyroidectomy, although an immediate postoperative increase has been noted by Dobyns[3] and others. Thyroidectomy usually has the opposite effect on progressive exophthalmos, increasing the proptosis and often leading to acute embarrassment of orbital circulation, ocular motility, and even of vision itself.

There has been a tendency in the past to consider that 2 different mechanisms

are involved in the production of these 2 apparently different types of exophthalmos. From recent clinical and experimental investigations, however, it would appear more logical to assume that the exophthalmos does not differ fundamentally in type but only in course and degree, and that this difference in course and degree depends upon the presence or absence of a compensatory mechanism for the control of the production of an exophthalmos stimulating substance. It seems likely that the pituitary produces the substance that is responsible for exophthalmos and that the substance for its control is elaborated in the thyroid. Originally it was thought that both the retraction of the lids and the proptosis might be explained on the basis of sympathetic stimulation brought about by excess secretion of thyroxin. Sympathetic stimulation causes exophthalmos in certain animals, but this mechanism is undoubtedly not active in man. Increased sympathetic tone may well account for lid retraction alone; but, as shown by Pochin,[10] exophthalmos cannot be produced in man by sympathetic stimulation. It has been known for many years that extracts of the anterior pituitary, containing thyroid stimulating hormone, cause exophthalmos. According to Means,[7] this was first demonstrated by Schockaert in 1931 and has been amply confirmed and elaborated upon. Pituitary extracts generally produce an initial rise in metabolism accompanied by exophthalmos. This exophthalmos is enhanced by thyroidectomy, but animal experimenters have never produced exophthalmos which is exactly comparable to the severe progressive type seen in man.

Long continued exophthalmos produced in animals by pituitary extracts is reported to lead to permanent changes in the extra-ocular muscles according to Aird.[1] Other factors are also of importance in experimental exophthalmos. Marine believes that the gonads seem to play a rôle, for castration lessens the exophthalmos produced by thyrotropic hormone in certain animals and testosterone brings it back. Recent work by Dobyns[3] has shown that thyroidectomy alone causes an increased prominence of the eyes of guinea pigs. Smelser[14] demonstrated that the exophthalmos produced by thyrotropic hormone in thyroidectomized guinea pigs seems to be due chiefly to an increased water content of orbital fat and extra-ocular muscles and that this same change is not noted in muscle or fat of other parts of the body. Neither thyroidectomy alone nor thyrotropic hormone produced this result. Rundle and Pochin[11] found marked increase of the fat content of the extras ocular muscles in human exophthalmo-associated with Graves' disease. These experimental studies would seem to indicate that the pituitary thyrotropic hormone is the agent responsible for the production of exophthalmos. The rôle that the thyroid plays as a controlling factor in the production of this hormone seems also to have been shown. From the clinical standpoint the recent works of Means[7] and Mann[5] would seem to substantiate these conclusions while the work of Mulvany[8] seems opposed at least in part.

Mulvany, along with others, believes that the exophthalmos in exophthalmic goiter is due to an overactive sympathetic nervous system. He explains retraction of the lids as due to stimulation of Müller's tarsal muscle and exophthalmos as due to the combined pull of Müller's and Landström's muscles opposed by weakened extra-ocular muscles. Mulvany states that these 2 sets of unstriated muscles exert a direct and an indirect pull on the globe, and that the weakness of the voluntary extra-ocular muscles is due partly to thyrotoxic myasthenia and partly to atonia caused by local neuromuscular degeneration. That sympathetic overactivity is the rule in hyperthyroidism cannot be doubted, but it seems that undue emphasis is placed upon the very small essentially vestigial unstriated muscles in the orbit as they exist in man. In progressive exophthalmos, Mulvany[8] states that retraction of the lids is due to enlargement of the levator muscle and that

exophthalmos is the result of an increase of the orbital contents. This author describes different microscopic findings in the 2 varieties of exophthalmos. In cases of exophthalmic goiter of the usual type he finds widespread neuromuscular degeneration, occasional infiltration of round cells, fat infiltration, and some fibrous replacement in the disintegrating muscle and nerve cells. In malignant exophthalmos he finds enlargement of the muscles, fibrosis which is often widespread through the muscles, edema, degeneration, and round cell infiltration. This author emphasizes the contrast between the 2 processes but, as Mann states, it seems possible that one could be an early and the other a late and more extensive phase of the same pathologic process.

In contrast to the hypothesis advanced by Mulvany, Means,[7] and Mann[5] believe that probably these 2 types of exophthalmos have a single basis. This basis is the production of excessive amounts of thyroid stimulating hormone by the pituitary perhaps initiated, as Means states, by nervous stimulation through the hypothalamus. On this basis, this excessive secretion of thyrotropic hormone by the pituitary causes the normal thyroid to respond with hypertrophy and excessive secretion of thyroid hormone and initiates the production of exophthalmos. Along with these changes there is present a certain degree of sympathetic hypertonia and of myasthenia, perhaps on the basis of concomitant alterations in the adrenal and thymus or perhaps due to the excessive thyroxine alone. The excessive thyroid hormone, besides stimulating metabolism, acts as an inhibiting mechanism on the pituitary causing lessened production of thyrotropic hormone. Thus the exophthalmos does not become excessive because the thyrotropic hormone, although produced in a supernormal amount, is controlled by a regulatory mechanism. The degree of exophthalmos present may vary from moderately severe in some cases to none in others; the latter cases exhibiting only lid retraction and perhaps some weakness of the ocular muscles. An intractable progressive type never results. In the case of progressive exophthalmos the excessive amount of thyrotropic hormone is produced as before; but, instead of stimulating normal thyroid tissue, for some unknown reason the thyroid in these cases seems unable to respond normally with the production of large amounts of thyroid hormone. Uninhibited by excessive hormone from the thyroid the pituitary goes on to secrete still more thyrotropic hormone. The exophthalmos continues to increase and when the process is of long enough duration and sufficiently severe, the pathologic changes present in the ocular muscles become irreversible. There are obviously a number of suppositions in this hypothesis. Just where the abnormality is present in the usually self-regulatory mechanism is open to question, but some recent works with thiourea derivatives may give a clue. Thiouracil apparently exerts its action by interfering with the production of thyroid hormone by the thyroid gland, and the resulting deficiency of thyroid hormone directly or indirectly stimulates the anterior lobe of the pituitary. The increased secretion of thyroid stimulating hormone by the anterior lobe induces thyroid hyperplasia.[4]

Means states that hyperplasia of the thyroid may be obtained in rats by treatment with sulfathiazole and exophthalmos results which persist after death. In man, sulfocyanate therapy may cause hyperplasia of the thyroid with hypofunction and the same author states that exophthalmos may occur. The following is a quotation from Means relative to the production of exophthalmos in these cases: "It is not clear precisely where such agents impinge upon the pituitary-thyroid axis, but the weight of evidence is that it is on the thyroid directly, imposing some obstruction to the completion of the elaboration of thyroid hormone. The organism thus becomes hypothyroid, which serves as a stimulus to the pituitary to increase its output of thyrotropic hormone, which, in turn, causes the thyroid to become hyper-

plastic and the eyes to become protruded. The hyperplasia of the thyroid, however, is ineffective because the obstruction to completion of thyroid hormone persists." It may be that such an obstruction to thyroid hormone production exists in all cases of progressive exophthalmos, the degree of obstruction determining the degree of hypo- or hyperthyroidism present.

In Mann's recent work she has presented a series of case reports in an attempt to disentangle the parts played by the thyroid and pituitary, respectively, in individual patients. The cases are divided into 3 groups on the basis of their most prominent symptoms. The first group contains patients who show primarily a deficiency of thyroid hormone with a compensatory excess of thyrotropic hormone. In the second group the cases show a primary excess of thyroid hormone, which, later, as the result of thyroid atrophy or removal is replaced by excess thyrotropic hormone. The third group shows symptoms of excess thyroid hormone and excess thyrotropic hormone arising simultaneously. On the basis of this study Mann concludes that the signs of excess thyroid secretion include loss of weight, rapid pulse, raised B.M.R., moist skin, lid retraction, and lid lag. Excess thyrotropic hormone leads to symptoms consisting of orbital muscle, lid, and conjunctival edema and infiltration which results in proptosis, ophthalmoplegia and fundus changes. Lid edema, chemosis and eversion of the conjunctiva are also the result of the orbital edema. All of these latter changes may or may not be associated with a deficiency of thyroid hormone. If this is present, it will show itself as low B.M.R., a gain in weight, thick coarse skin and slow pulse.

Agreement is by no means reached as to treatment of these obstinate cases of exophthalmos. The management of these cases of progressive exophthalmos taxes the ophthalmologists resources to the utmost and any advance in therapy is eagerly sought after. Attempts to treat the condition from an etiologic standpoint are still in their infancy and reports are insufficient to evaluate their true position at the present time. Thyroid hormones in large doses has been used with fair success, the beneficial results supposedly coming from inhibition of pituitary secretion. In the article by Mann thyroid therapy and surgical procedures designed to close the lids were used in these cases which did not exhibit marked hyperthyroidism. The end-result in these cases was exceptionally gratifying. Attacking the problem of reducing the pituitary secretion, Brain[2] has used ovarian follicular hormone in large doses with good success. He gives 50,000 units of progynon B every 5th day to both sexes and states that "exophthalmos usually diminishes." He reports that 1 early case of exophthalmic ophthalmoplegia made a complete recovery under this treatment. Direct irradiation to the pituitary in order to reduce its secretion has been tried by several investigators, including those at the Mayo Clinic, with rather disappointing results. We have attempted treatment with thyroid hormone and iodine combined in an effort to maintain the basal metabolism at a normal level. Results somewhat less striking than those shown by Mann have been obtained. Agreement, however, is almost universal concerning one point in the treatment of these cases and this point is the inadvisability of performing thyroidectomy. The group of cases presenting early symptoms of progressive exophthalmos must be weeded out of routine hyperthyroid patients if disastrous results are to be avoided. This point is especially stressed by Salter and Soley,[12] and by Means. The former authors recommend Roentgen ray therapy to the thyroid if symptoms of hyperthyroidism are prominent and if the condition of the patient demands treatment along this line. Thiouracil and radioactive iodine have also been advocated in treating this group of patients. Many authors advise decompression operations on the orbit in the severe cases of exophthalmos especially when the vision is at stake. The Naffziger operation is

the one most frequently employed but it cannot be undertaken lightly for the operation still remains a somewhat formidable procedure. Recently an alternative operation has been advocated by Schall and Reagan[13] who perform a radical external ethmoidectomy. They believe that this operation should be carried out more frequently and observed, in their cases, a reduction of exophthalmos of 3 mm. They state that, opposed to the Naffziger procedure, their operation is less formidable, produces a less disfiguring scar, creates an actual rather than a potential space, and does not result in a pulsating globe. Decompression operations of these types usually are not advocated until it has become obvious that the exophthalmos is progressing to a dangerous point in spite of adequate medical management.

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PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF DECEMBER 15, 1946

A New Concept of Competitive Inhibition of the Renal Tubular Excretion of Penicillin. KARL H. BEYER, PH.D., M.D., HORACE F. RUSSO, B.S., ELIZABETH A. PATCH, B.S., and A. KATHRINE MILLER, PH.D. (Depts. of Pharmacol. and Bact., Med. Res. Div., Sharp & Dohme, Glenolden, Pa.). It has been established that penicillin is excreted both by the renal tubules and by glomerular filtration. The renal clearance of penicillin is normally about 5 times glomerular filtration rate.

The tubular transport mechanism for penicillin, p-aminohippurate (PAH) and Diodrast excretion is functionally the same. It has been reported that on a mass action basis one could competitively inhibit the tubular excretion of penicillin by saturating with PAH at high blood levels the functional capacity of the mechanism.

Caronamide, 4′ - carboxy - phenylme - thanesulfonanilide,* effects a substantital physiologic economy of penicillin on an essentially different basis. It appears that the mode of action of this agent is to halt the transport mechanism responsible for the tubular excretion of penicillin and PAH.

Certain of the pharmacologic characteristics of caronamide are: (1) It is rapidly absorbed when administered orally, and may be given by this route to inhibit the tubular excretion of penicillin administered orally or parenterally. (2) Its renal elimination is limited essentially to glomerular filtration. (3) Its action is to inhibit the excretory transport mechanism *per se* for the tubular excretion of penicillins F, G, K and X, and PAH. (4) The effect of the agent appears to be limited to a functionally single transport mechanism, for caronamide does not influence the Tm of glucose or arginine, the clearance of creatinine, urea, sulfadiazine, sulfathiazole, or the kidney volume of dogs at blood levels sufficient to suppress completely the tubular excretion of penicillin. (5) The compound has a low order of acute and chronic toxicity.

The efficacy and safety of caronamide have been substantiated clinically.

The Hyperpnea Produced in Normal Man by Maximal Voluntary Hyperventilation, by Inhalation of CO_2 and by Severe Muscular Exercise. J. H. COMROE, JR., M.D., and ROBERT D. DRIPPS, M.D. (Depts. of Pharmacol., Surg. Res., Anesthesiol., Med. Sch.; Physiol. and Pharmacol., Grad. Sch. Med., Univ. of Penna.). These experiments were designed to measure and compare (*a*) the maximal minute volume of respiration of which man is capable by voluntary effort (the maximal breathing capacity), (*b*) the maximal minute volume produced by CO_2 inhalation, and (*c*) that produced by severe muscular exercise in normal physically fit young men. The results of these experiments are shown in Table 1.

So far as the CO_2 inhalations are concerned the data show marked individual variations from subject to subject which is presumably due to differences in sensitivity of the medullary respiratory centers. Inhalation of 10.4% CO_2 produced maximal ventilation which was only 46% of the subjects' total breathing capacity. It is probable that CO_2 inhalation might have led to greater

* Sharp & Dohme has applied its trademark "Retentin" to this compound.

hyperpneas if certain inhibitory factors were not acting. These factors include inhibition of respiration (*a*) arising from the pressure receptors of the carotid sinus and aortic arch in response to the hypertension produced concomitantly and (*b*) due to narcosis of the centers by a direct depressant effect of high concentrations of CO_2 (of the 31 subjects breathing 10.4% CO_2, the following symptoms suggestive of central nervous system depression occurred: dizziness in 13, faintness in 7, unconsciousness in 3, symptoms resembling induction of anesthesia with nitrous oxide in 2, and analgesia in 1).

Table 1

Procedure	*Subjects*	*Maximum average*	*Maximum range*	*Std. dev.*	*%*
Maximal breathing capacity	19	166	132–198	20.3	100
CO_2 7.6%	42	52	24–102	18.4	31
CO_2 10.4%	31	76	40–130	24.8	46
Muscular exercise	19	110	80–140	18.3	66

Muscular exercise produced more hyperpnea in 18 of 19 subjects than did inhalation of CO_2. The small changes in arterial CO_2 tension occurring in muscular exercise cannot account for the marked hyperpnea of muscular exercise.

The minute volumes at which dyspnea occurred in subjects breathing CO_2 were noted. In the group that experienced no dyspnea, maximal minute volumes ranged from 24 to 114 liters per minute (average 60); in those who experienced mild to moderate dyspnea, the minute volumes were 29 to 110 (63) and in those who noted marked dyspnea, the minute volumes were 50 to 130 (87). This emphasizes the fact that dyspnea is a subjective phenomenon not necessarily related to the volume of respiration. Some subjects did not notice hyperpnea in themselves until ventilation reached 30 to 40 liters per minute. It is therefore often desirable to measure both rate and minute volume of respiration in certain clinical cases.

Variation in Protein and Polysaccharide Content of Sera in Tuberculosis, Sarcoidosis, and Carcinoma. Florence B. Seibert, Ph.D., Mabel V. Seibert, A. Jane Atno, A.B., and Harold W. Campbell, M.D. (Henry Phipps Inst., Univ. of Penna.). Electrophoretic analyses of sera, combined with chemical analyses for polysaccharide, can be helpful in the differential diagnosis of tuberculosis, sarcoidosis and carcinoma. Cases of tuberculosis were carefully selected in the clinic according to their stage of the disease. The carcinoma cases included lesions of many different organs, and 4 of the sarcoidosis series were verified by study of biopsy material. At least 20 cases of each type of disease were studied and the following conclusions were based upon the statistical analysis of the results.

Significant changes occurred in certain of the constituents of the blood, which appeared characteristic for the different diseases or different stages of disease. Some of the most conspicuous of these changes were the following: A rise in the gamma globulin occurred in the serum in minimal active tuberculosis, with a corresponding decrease in albumin. In moderately advanced disease the α_2 globulin and polysaccharide content also increased. These changes were still further emphasized in far-advanced tuberculosis and the other globulins were also increased. No deviation from the normal occurred in minimal tuberculosis of questionable clinical significance. Moderately advanced tuberculosis of questionable clinical significance showed only a decrease in albumin, indicating that it was the last component to return to normal. Sera from cases of sarcoidosis showed an increase in total protein, a proportionate large increase in gamma globulin and only a moderate increase in polysaccha-

ride content. In carcinoma there was a decrease in total protein, a large increase in α_2 globulin and beta globulin and polysaccharide content.

It is suggested that the increase in gamma globulin in minimal tuberculosis may indicate antibody formation, whereas the rise in α_2 globulin and simultaneous rise in polysaccharide in advanced tuberculosis and carcinoma may represent tissue destruction.

The Non-participation of 1(+) Citrulline as an Intermediate in the Synthesis of Urea by Rat Liver Slices. JOHN H. NODINE and JOHN M. BUCHANAN, PH.D. (Dept. of Physiol. Chemistry, Univ. of Penna.). Since Krebs and Henseleit (*Ztschr. f. physiol. Chem.*, **210**, 33, 1932) postulated the "ornithine cycle" as the main metabolic pathway of urea formation, numerous investigations have been undertaken to substantiate or disprove this hypothesis. The purpose of this present study is to test the hypothesis directly using isotopes. Rat liver slices were incubated in a medium containing isotopic bicarbonate (C^{13}) buffer, NH_4Cl, dl-ornithine and other ionic constituents as described by Krebs, both in the presence and absence of large amounts of 1(+) citrulline. The urea synthesized contained 2.08 and 2.19 atoms % excess C^{13} with and without citrulline respectively. The citrulline isolated at the end of the reaction showed no excess C^{13}. Evidence for adequate mixing of intra- and extracellular citrulline was presented by Krebs and others who showed an acceleration of urea formation (an intracellular process) upon the addition of citrulline to a medium similar to ours, and Gornall and Hunter (*J. Biol. Chem.*, **147**, 593, 1943), who showed that citrulline accumulates in a medium containing liver slices and ornithine, the former showing diffusion into the cell, the latter showing diffusion out of the cell. It was thus concluded that 1(+) citrulline is not a major intermediate in the synthesis of urea from CO_2 and NH_3 in rat liver slices under the conditions of our experiments. Calculations from the C^{13} concentration of the medium showed that 90 to 100% of the carbon of the urea formed was derived from the CO_2 of the medium. Thus the added citrulline was not converted to urea to any significant extent under the conditions of our experiments. A 3-fold acceleration of urea formation was noted upon addition of the citrulline to the reaction mixture. It was concluded that under the conditions of our experiments urea was not synthesized by the "ornithine cycle" as presently postulated.

The Effect of Inhalation of High and of Low Oxygen Concentration Upon Human Respiration and Circulation. ROBERT D. DRIPPS, M.D., and JULIUS H. COMROE, JR., M.D. (Depts. of Pharmacol., Surg. Res., Anesthesiol., Med. Sch.; Physiol. and Pharmacol., Grad. Sch. Med., Univ. of Penna.). Sixty-eight normal human subjects were permitted to breathe some of the following: 100% oxygen, 20.9, 18, 16 or 14.5% oxygen in nitrogen. Pulse rate and respiratory minute volume were recorded. In 28 of 33 subjects studied during the change from room air to 100% oxygen, a small immediate decrease in respiratory minute volume or pulse rate was observed. Average respiratory minute volume was depressed 3.2% and pulse rate 3.7% during the first 2 minutes of 100% oxygen inhalation. Statistical analyses of these data revealed a likelihood that the respiratory changes might have occurred by chance in 1 of 15 instances, a figure below the 1 in 20 normally regarded as significant in medical statistics. The possibility of the pulse rate changes occurring by chance was less than 1 in 1000, a highly significant observation.

If this change can be attributed wholly to a functional denervation of oxygen-sensitive receptors in the carotid and aortic bodies, these data suggest that

some chemoreceptors are tonically active at the oxygen tension present in the arterial blood of many normal men breathing room air at sea level. The small degree of the changes indicated that they are only minimally active.

Fourteen subjects breathed 18% oxygen and 21 breathed 16% oxygen after control data on expiratory minute volume and pulse rate had been obtained with room air. An increase in pulse rate was a more sensitive index of anoxemia than was an increase in respiratory minute volume. Of the subjects, 79% showed an increase in pulse rate with 18% oxygen, and only 36% showed increased ventilation (5% increases above control values).

Our data indicate that the human circulatory system is usually stimulated by reducing the inspired oxygen percentage from 20.9 to 18, but respiration is stimulated in only a few individuals breathing 18 or 17% oxygen and is not increased in the majority until 16% oxygen is inhaled.

BOOK REVIEWS AND NOTICES

NUTRITION AND CHEMICAL GROWTH IN CHILDHOOD. Vol. II. Original Data. By ICIE G. MACY, PH.D., SC.D., Director of the Research Laboratory, Children's Fund of Michigan. With a Supplement by JULIA O. HOLMES, PH.D. Index to Vols. I and II. Pp. 1044; 1081 ills. and figs. Springfield, Ill.: Thomas, 1945. Price, $10.00.

FOUR years ago Dr. Macy and his associates presented Vol. I of Nutrition and Chemical Growth in Childhood, in which were stated the conditions under which the 10 year study was conducted, the methods employed to secure the samples and data and their statistical treatment.

Vol. II, titled "Original Data," may be divided into 3 parts: the data obtained by the Research Laboratory of the Children's Fund of Michigan, similar data obtained by Dr. Julia Holmes (formerly associated with Dr. Macy) at The Department of Home Economics of the University of Illinois on pre-school children, and comparative data on certain abnormal cases studied by the Michigan group by their research methods which were referred by the Children's Hospital.

Within the confines of a necessarily brief review it is impossible to analyze and evaluate the mass of data here presented. Inaccuracies and incongruities will be pointed out by future workers in these fields. Certainly very few typographic or gross errors are apparent.

The original material consists of thousands of data obtained in the course of studying serially a number of normal children living at the Methodist Village. These data are concerned with the foods ingested, and analyses of urine and feces. For each child at each period of observation there are also records of physical examination, psychologic and endocrine estimations, anthropometric measurements, basal metabolism, activity, laxation rates, emotional reactions, blood studies and dental conditions, etc., which are correlated with the balance studies. Even the climatologic conditions have been recorded.

One feature of these observations which when assembled would comprise a valuable monograph is the presentation of serial growth roentgenograms of the bones, the teeth and the gastro-intestinal tract following the use of various test meals. The reproductions of these films are amazingly clear.

If one could point to any aspect which is below the average in value and excellence, this Reviewer would suggest that the psychologic and so-called endocrine studies add little to the mosaic and are not of equal value.

About 60 pages are devoted to the studies of Dr. Holmes and the Illinois group on 5 girls who were studied for 32 consecutive weeks, and on 7 boys studied for 72 weeks. Nitrogen, phosphorus and calcium balances related to growth and skeletal structure were the special objects of these studies.

A prominent feature of the "Original Studies," expanded in the Addenda is the assemblage of data on the blood in health and disease.

In the course of Dr. Macy's work, certain disease conditions well suited for study by the methods used were admitted to the Children's Hospital. These cases are also presented in the Addenda and include Osteopsathyrosis, ununited fracture, lipemia and epilepsy.

The material in this volume should prove authoritative and valuable for workers in growth or nutrition as well as chemistry. They should supplant data which have been obtained from animals. The data would seem to be especially timely in view of the nutritional conditions which exist and must be corrected in a scientific way in the war-torn countries of Europe and Asia. Expected results of food relief can be based on and compared with the work of Dr. Macy.

E. T.

PREVENTIVE MEDICINE AND PUBLIC HEALTH. By WILSON G. SMILLIE, M.D., Professor of Public Health and Preventive Medicine, Cornell University Medical College. Pp. 607; 41 ills. New York: Macmillan, 1946. Price, $6.00.

THIS volume is written from the point of view that preventive medicine is an essential part of the practice of medicine. Care-

ful examination of the text reveals the clear, concise and comprehensive manner in which the author has fulfilled this aim.

Teachers, students and physicians who have been in the habit of going to this author's volume "Public Health Administration in the United States" for general information in the field, and especially to find a terse, satisfying, opinion upon controversial matters, will find the same dependable quality here.

In his Preface the author admits that "the whole broad field of the social aspects of medical care has been presented most inadequately." This apparent modesty must not be misunderstood; it is actually a result of his exhaustive and persistent literary and personal research undertaken to find practical and intelligent solutions for matters which "are still a fluid concept that is not ready for presentation in textual form." Nevertheless, the chapter on "Adequacy of Medical Care" is a superior production.

The text is dedicated to Lemuel Shattuck, a Boston bookseller whose studies in vital statistics of New England aroused his concern for public health and preventive medicine. His report, published in 1850, became a foundation stone upon which even our current programs might well be built. Each section of this volume is introduced by a remarkably modern quotation from Shattuck's report.

The book is an attractive one both in appearance and in diction. It can be recommended as a dependable guide for the physician, the student and the public health official.

A. H.

SYNOPSIS OF NEUROPSYCHIATRY. By LOWELL S. SELLING, SC.M., M.D., PH.D., DR.P.H., Director, Psychopathic Clinic, Recorder's Court, Detroit, Mich.; Associate Attending Neuropsychiatrist, Eloise Hospital; Adjunct Attending Neuropsychiatrist, Harper Hospital. Pp. 500. St. Louis: Mosby, 1944. Price, $5.00.

THE new book shelves of our medical libraries have been carrying an ever increasing number of volumes with such titles as psychiatry, neuropsychiatry, physiologic psychology, medical psychiatry, mental disorders, mental hygiene, psychopathic personality, psychosomatic medicine, etc.

While one would hardly question an author's rationale in limiting the field of discussion under his particular title, there is little doubt that each author does differ in his own conception to a degree that gives rise to a great variety of meanings and interpretations based on his own training and experience. This trend has led to enough confusion in this field to excite comment at the Centennial Meeting of American Psychiatric Association in Philadelphia. A member arose and asked for the formation of a committee to establish definition of terms that would find greater agreement amongst a wider variety of workers in the field. While small active groups in confined areas may understand each other, the less active (in research) and more remote by location have found great difficulty in understanding the newer connotations of the language. Nothing apparently came of the proposal.

While reviewing the "Synopsis" it occurred to the Reviewer that here was a book that can, more or less, do what the "Unformed Committee" may have accomplished.

The "Synopsis" provides a means of attaching oneself to the basic concepts with which newer ones can be compared or associated. The elements of neuroanatomy and neuropathology described serve as a basis of understanding the organic neurologic syndrome discussed. An extensive review of psychopathology, abnormal psychology and psychoanalysis presents a fine background for functional disorders.

The book is divided into 2 parts: Neurology, 10 chapters, with a total of 296 pages, and Mental Disorders, 15 chapters, totaling 177 pages. The classification of personalities is given a modern touch in describing such personalities as "Caspar Milquetoast," "wolves," "fairies" and "screwballs."

J. S.

SYNOPSIS OF PHYSIOLOGY. By ROLLAND J. MAIN, PH.D., Professor of Physiology, Medical College of Virginia. Pp. 341; 21 ills. St. Louis: Mosby, 1946. Price, $3.50.

THIS small book aims to be a text for review purposes for students with some knowledge of physiology and of medicine. The book covers a wide field in an extremely brief form and is clearly expressed. There are relatively few figures but these are very clear diagrammatic presentations of material. The book is likely to have a considerable use by students who want to revise for

examinations. In this respect it will be very useful.

Apparently clinicians also request "a little book which hits the high spots." The Reviewer believes that such use would be a retrograde step in education. Knowledge should be used and be integrated into ever more comprehensive patterns. It is the thinking about interrelated facts that is important rather than the memorizing of such facts. The author has unquestionably thought considerably, but the brief statement does not encourage the reader to go beyond the author and to utilize his own imagination. Consequently the Reviewer finds himself diametrically opposed to the principle of such a book for use of medical graduates. H. B.

DIAGNOSIS AND TREATMENT OF PULMONARY TUBERCULOSIS. By MOSES J. STONE, M.D., Assistant Professor in Medicine, Boston University School of Medicine; Instructor in Medicine, Tufts Medical School; Physician-in-Chief, Chest Clinics, Beth Israel and Massachusetts Memorial Hospitals; and PAUL DUFAULT, M.D., F.A.C.P., Superintendent of the Rutland State Sanatorium, Rutland, Mass. First 2 editions by Hawes and Stone. Pp. 325; 93 engravings. Philadelphia: Lea & Febiger, 1946. Price, $3.50.

BECAUSE of the decreasing number of physicians and students interested in prolonged training in tuberculosis, there is need for a concise, comprehensive text on basic concepts and treatment of pulmonary tuberculosis. The authors of this book have produced an easily read and lucid guide in diagnosis and treatment. It is to be recommended to medical students and general practitioners as an elementary treatise in phthisiology. For those interested in broader training in this field a supplemental list of suggested reading is appended at the conclusion of each chapter.

Certain improvements could be made in the section on specific therapy. A treatise on this subject in 1946 might well include at least brief mention of streptomycin. In discussing the treatment of laryngeal tuberculosis, where so little can be offered the patient, the promising results of sulfa drug and penicillin insufflation should be noted. The sections on collapse therapy should have discussed pneumoperitoneum as an adjunct in therapy and not merely as a possible complication of artificial pneumothorax.

The arrangement of the chapters is good, and the authors have accomplished their object in commendably short space. S. S.

THE BIOLOGY OF SCHIZOPHRENIA. By R. G. HOSKINS, PH.D., M.D., Director of Research, Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School and Worcester State Hospital. Pp. 191. New York: Norton, 1946. Price, $2.75.

IN reference to the vast amount of human distress and of the unproductive expense resulting from schizophrenia, it is said one-fifth of all hospitalized patients in the country and almost 10% of state tax (Massachusetts) is required to care for the victims of this disease. Continuing the study of the condition as one of disordered biology, the writer discusses his thesis in the following sections: The Biology of Man in Relation to Schizophrenia; The Pattern of Schizophrenia; Psychosomatic Aspects of Schizophrenia; A Biological Appraisal of Schizophrenia.

These distorted personalities are most apt to develop in inadequate and frustrated people. As to cause—there is the possibility "that adequate studies of the enzymes might disclose a genuine pathology for the psychosis." After studying the nature of man, the pattern of the disorder is compared with that of other biologic patterns; and finally there are considered the chief functional deviations frequently observed in the endocrines, oxygen and carbohydrate metabolisms, and the circulatory system.

The writer's approach to the subject is unusual and stimulating. N. Y.

DIGITALIS AND OTHER CARDIOTONIC DRUGS. By ELI RODIN MOVITT, M.D., CAPT., M.C., A.U.S.; Internist, Veterans Administration Facility, San Francisco. Pp. 204; 24 ills. New York, Oxford Univ. Press, 1946. Price, not given.

THE subject matter of this book includes digitalis leaf (purpurea) and various glycosides, digitalis lanata and lanatoside C,

strophanthus and its derivatives, squill and various other so-called cardiotonic agents of plant and animal origin. Historical data, source and chemical structure, pharmacology and use in clinical treatment are discussed.

The literature has been carefully reviewed and the book may be regarded as a dependable source of information with respect to digitalis and allied drugs. C. W.

MEDICINE IN INDUSTRY. By BERNHARD J. STERN, PH.D., Lecturer in Sociology, Columbia Univ.; Visiting Professor of Sociology, Yale University. Pp. 209; 15 tables. New York: Commonwealth Fund, 1946. Price, $1.50.

THIS is one of the volumes issued under the auspices of the Committee on Medicine and the Changing Order of the New York Academy of Medicine, "as a contribution to contemporary thought on important questions in the general medical and health field." The author is well known as a writer upon topics which are closely allied to medicine and his selection by the committee assured a thoroughly documented and critical survey to demonstrate how perfectly this particular subject illustrates "the reciprocal effects of medicine and the technical, social, economic, and political changes that have taken place in American life."

Each of the 7 chapters represents a review of the subject under discussion both historically and currently. Appended to these chapters are references to all the important publications a student or practitioner is likely to need. The first 2 chapters, scientific developments and social and legislative backgrounds, take us from a papyrus of ancient Egypt to the most recently published laws enacted by various states up to last year.

Progress has been spotty. In all ages the need for reform has been recognized but it is only in recent years that there has been satisfactory achievement. Attainment of ideal conditions still depends on research, especially in the field of physiology. In considering the extent of industrial disability it is found that the data available do not approximate accuracy but that they are sufficient to permit an evaluation of the prevailing situation.

The study here of the handicapped worker in industry reveals a problem which the war disabilities may help to solve by requiring a more constructive and enlightened policy on their employment than is now generally in force.

Preventive services which can be standardized, methods, and the extent of their utilization, are the result of intensive organization. It is now in the smaller plants that safety services are less available. There are compelling facts in the figures assembled under health programs and financial savings.

Medical care programs in industry are financed by employers and workers jointly, by workers alone or by employers alone. The review here of the current utilization of plans for health insurance in industry furnishes a perspective which can be used by individuals, groups of employers or communities for their own projects. Probably nowhere else can there be found a summary so well balanced.

The final chapter concerns the industrial physician; it states the conventional functions of those who are considered specialists. With the recognition of psychiatry as an aid in placement, avoidance of accidents and absenteeism, the alert industrial physician finds himself called upon to fulfil many other important functions. He will be a member of the safety committee, a consultant of management and probably personnel director. "The field of industrial medicine offers one of the most fruitful approaches to preventive medicine and public health. As the schools are agencies through which children can be reached *en masse* for the application of the most advanced findings of scientific medicine, so the factories, workshops and commercial establishments afford the opportunity to reach large aggregations of adult workers."

In an Appendix there is a tabulation of certain industrial prepayment medical plans. Organization of the oldest of these, the Hospital Department, Southern Pacific Company, dates back to 1867.

This small but important volume traces "the social, economic, legal, and professional setting within which industrial medicine has matured and the development of the scientific knowledge which has given industrial physicians increased competence to cope with diseases affected by occupations."

A. H.

PSYCHOLOGICAL MEDICINE. By DESMOND CURRAN, M.B., F.R.C.P., D.P.M., Psychiatrist and Lecturer in Psychological Medicine, St. George's Hospital, and Honorary Psychiatrist to the Midia Vale Hospital for Nervous Diseases, London; Temp. Surgeon Captain, R.N.V.R., and Consultant in Psychological Medicine to the Royal Navy; and ERIC GUTTMANN, M.D., M.R.C.P., Neuropsychiatric Specialist, Emergency Medical Service; formerly Research Psychiatrist, Nuffield Dept. of Surgery, Oxford; Psychiatrist to the Officer Board, National Fire Service. Foreword by J. J. CONYBEARE, M.C., D.M. (OXON.), F.R.C.P., Physician to Guy's Hospital, London. 2nd ed. Pp. 246; 20 ills. Baltimore: Williams & Wilkins, 1945. Price, $3.50.

THE main changes and additions found in this issue are concerned with constitutional factors and psychopathic personalities, the affect and hysterical syndromes, and modern physical methods of treatment. Important chapters are: Ætiology of Mental Disorder; Symptoms in Mental Disease; Psychiatric Case - Taking; Treatment; Constitutional Anomalies; Organic Syndromes; Psychiatric Aspects of Head Injury; Drug Addictions; Schizophrenia; The Affect Reaction Types; Obsessional States; Hysterical Reactions. The appended Psychiatry Associated With War Conditions is enlarged.

Psychosomatic Medicine is now included with 4 distinct avenues of approach: The study of the psychologic origin of somatic symptoms and of organ neuroses; the discovery of psychologic factors precipitating or causing organic disease; somatic disease in relation to psychologic types; psychologic reactions to somatic disease and defect.

N. Y.

PROGRESS IN NEUROLOGY AND PSYCHIATRY. Edited by E. A. SPIEGEL, M.D., Professor and Head of the Department of Experimental Neurology, Temple University School of Medicine. Fifty-three contributors. Pp. 708. New York: Grune & Stratton, 1946. Price, $8.00.

IN this collaborate volume, the initial one in forthcoming annual reviews, the subject-matter is confined largely to the literature of 1945. Most space is given to Pharmacology of the Nervous System, where the biography alone covers more than 23 pages; its chief concern is in diagnostic and therapeutic applications, with but little consideration of drug action mechanisms. Among other subjects ar general and local anesthetics; the various aspects of alcoholic addiction, including the efforts of Alcoholics Anonymous and the Yale Plan Clinics; in the war neuroses, ergotamine tartrate has been employed in overcoming "sympathetic overactivity." In shock therapy, affective disorders respond much more readily than does the schizophrenic group. Electro-encephalograms show moderately severe "abnormalities" appearing in from 5 to 15% of the clinically "normal" control subjects. When penicillin is given alone, as yet there is no standardized method for its use in neurosyphilis. Under Group Psychotherapy, psychodrama receives careful consideration: on a specially designed stage, the subject is encouraged to act out his problems before an audience of patients, visitors and staff. There is a scarcity of summaries. This product of many collaborators is a timely book on opportune subjects, and its annual re-appearance doubtless will be looked forward to.

N. Y.

STUDIES IN HYPERTONY AND THE PREVENTION OF DISEASE. By I. HARRIS, M.D., Honorary Director, Institute for Prevention of Disease, Honorary Physician, Liverpool Heart Hospital ; in coöperation with J. T. IRELAND, B.SC. (HONS.), A.I.C., Leverhulme Research Fellow, G. V. JAMES, M.SC., A.I.C., Maurice Stern Research Fellow, EDWARD CRONIN LOWE, M.B.E., M.B., B.S., Director, Pathological Department, Southport Infirmary, Honorary Clinical Pathologist, Institute for Prevention of Disease, C. E. VERNON, M.Sc., A.I.C., Research Fellow. Pp. 114. Baltimore: Williams & Wilkins, 1946. Price, $3.00.

THIS book is unusual. The introduction contains a bitter attack on the Medical Research Council, for adding calcium to the bread of wartime England, describing them as "dictatorship or priesthood . . . to hold their positions at all costs and by all means."

The author believes that emotional strain and diet are the cardinal factors in producing hypertony, or hypertension. Much original experimental and clinical data is presented

in sections dealing with calcium, cholesterol, protein intake, phosphorus and potassium. The author's discussions and conclusions are ingenious and thought provoking, but will not be accepted by all. The book is recommended primarily for students of hypertension and metabolic disease. J. G.

MANUAL OF TUBERCULOSIS. By E. ASHWORTH UNDERWOOD, M.A., B.Sc., M.D., D.P.H., Medical Officer of Health and Chief Administrative Tuberculosis Officer, County Borough of West Ham; Lecturer in the Royal College of Nursing; Fellow of the Royal Statistical Society. Pp. 524; 88 ills. Baltimore: Williams & Wilkins, 1945. Price, $4.50.

SELDOM has the subject of tuberculosis been condensed as well as in this small volume by Dr. Ashworth Underwood. Within its pages the reader will find an informative and accurate account of the etiology, pathogenesis and evolution of tuberculosis, its institutional, medical and surgical treatment, the disease as it occurs in children, and the large problem of rehabilitation, as well as a very useful discussion of the administrative measures employed in tuberculosis control, the epidemiology of the disease and its public health aspects and its relation to social medicine. Quite appropriately, the book ends with a chapter on tuberculosis and war, in which a concise analysis is made on the basis of recent English experience of the impact of total war on a country previously well organized for tuberculosis control. The author is conservative in his account of tuberculosis, not leaning to any one of the several conflicting theories on pathogenesis, but giving a factual account of present understanding, and frankly admitting our ignorance of certain phases of the development of the disease.

The book is well written, and excellently illustrated. A useful feature is an accurate, comprehensive summarization at the end of each chapter. The volume is recommended for all persons interested in tuberculosis, and particularly for undergraduate and graduate medical students who wish a comprehensive view of tuberculosis for incorporation in their general understanding of medicine.

E. L.

PSYCHOTHERAPY IN GENERAL PRACTICE. Report on an Experimental Postgraduate Course. By GEDDES SMITH, Associate, The Commonwealth Fund. Pp. 38. New York: Commonwealth Fund, 1946. Price, 25c.

IN an experimental effort to determine if doctors could be "taught to practice in their own offices the kind of medicine psychoneurotic patients need," 25 physicians in general practice attended a postgraduate course of 2 weeks duration at the University of Minnesota. As a course in psychotherapy, such complicated problems as deep-seated sexual dysfunction, were not attempted. From the results on 121 patients seen, it appears that a new outlook was given on medicine: "their attitude toward patients, their attitude toward disease, and their treatment of chronic illness." N. Y.

MEDICAL CLINICS OF NORTH AMERICA. July 1946. Mayo Clinic. Splenomegaly. Pp. 253. Philadelphia: Saunders, Price, $16.00 yearly.

THIS is an informative and absorbing number. The leading article reviews well the disease entities to be considered should the examiner palpate the spleen in an adult patient. The papers on headache, gastroscopy, acute abdominal pain and chancroid stress the importance of the history in making the proper diagnosis. Physicians who have not had the opportunity to prescribe some of the more recently available chemotherapeutic agents will find the presentation on thiouracil, streptomycin, penicillin in the treatment of syphilis and the newer sulfonamides and antibiotics in intestinal diseases succinct and specific.

The reviews of the treatment of headache and habitual abortion, the present status of thiocyanates in hypertensive disease, the increasing abuse of sedative drugs and the use of various kinds of insulin critically cover the practical problems of managing such patients. Several of the clinics on the therapeutic agents present too enthusiastically the curative potentialities of these drugs. Studies on series of over 100 cases with a similar number of controls on placebo therapy are in the minority. An example is that more than a minority of patients with chancroidal ulcers do not get complete healing on sulfathiazole. Many patients having

acquired bacillary dysentery in this and other parts of the world are not improved by sulfadiazine even on near-toxic dosages. Increasing emphasis should be given to the toxic manifestations of these drugs which are so frequently prescribed.

The article on the Problem of Blackout and Unconsciousness in Aviators is unusual in a clinical presentation. This simple concise exposition of the vast amount of experimental work done in this field since 1939 is timely. It gives the medical man an opportunity to learn about the human problems in aviation relating to the survival of airmen, how the physiologic mechanisms were studied, what solutions were brought forth and what problems are still unanswered.

The physiologic investigation accomplished in the marvelous medical research program coördinated by the Office of Scientific Research and Development should be summarized in the future Clinics of the appropriate institutions. J. H.

JEWISH LUMINARIES IN MEDICAL HISTORY. By HARRY FRIEDENWALD, M.D., D.H.L. (HON.), D.Sc. (HON.), Professor Emeritus of Ophthalmology, University of Maryland, and a CATALOGUE of Works Bearing on the Subject of the Jews and Medicine From the Private Library of Harry Friedenwald. Pp. 199. Baltimore: Johns Hopkins Press, 1946. Price, $3.00.

As indicated by the title, this volume consists of a brief discussion of Jewish luminaries in medical history, and a catalogue of works bearing on the subject of the Jew in medicine, from the private library of the author.

If there is ever need for a source of information on the accomplishments of the Jew in medicine, the short lecture of Dr. Friedenwald, and what he calls, "A modest but unique collection . . . which in a measure illustrates the history of medicine among the Jews," supplies a basis for this purpose. This volume, well organized and written, but only sparingly illustrated, is a worthy addition to the library of any medical bibliophile. The value of the book is especially enhanced by the notes interpolated in the catalogue. H. B.

PRINCIPLES OF HEMATOLOGY. By RUSSELL L. HADEN, M.A., M.D., Chief of the Medical Division of the Cleveland Clinic; formerly Professor of Experimental Medicine in the University of Kansas School of Medicine. Pp. 366; 167 ills. 3rd Ed. Philadelphia: Lea & Febiger, 1946. Price, $5.00.

THIS indeed thoroughly revised 3rd edition of a valuable book written, as Dr. Haden says, "to simplify the studies of disorders of the blood for the student and physician," makes a very welcome third appearance. The description "of the technique of bone marrow puncture and the study of bone marrow films" that has been added increases materially the value of this excellent and advisedly "simple discussion of the fundamental principles of hematology." E. T.

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